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Risk of survival, establishment and spread of Batrachochytrium salamandrivorans (Bsal) in the EU

EFSA Panel on Animal Health and Welfare (AHAW)

2018-04-30

EFSA Panel on Animal Health and Welfare (AHAW) , More , S & Sihvonen , L H 2018 , ' Risk of survival, establishment and spread of Batrachochytrium salamandrivorans (Bsal) in the EU ' , EFSA Journal , vol. 16 , no. 4 , e05259 . <https://doi.org/10.2903/j.efsa.2018.5259>

<http://hdl.handle.net/10138/298846>

<https://doi.org/10.2903/j.efsa.2018.5259>

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ADOPTED: 11 April 2018

doi: 10.2903/j.efsa.2018.5259

Risk of survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU

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Abstract

Batrachochytrium salamandrivorans (Bsal) is an emerging fungal pathogen of salamanders. Despite limited surveillance, Bsal was detected in kept salamanders populations in Belgium, Germany, Spain, the Netherlands and the United Kingdom, and in wild populations in some regions of Belgium, Germany and the Netherlands. According to niche modelling, at least part of the distribution range of every salamander species in Europe overlaps with the climate conditions predicted to be suitable for Bsal. Passive surveillance is considered the most suitable approach for detection of Bsal emergence in wild populations. Demonstration of Bsal absence is considered feasible only in closed populations of kept susceptible species. In the wild, Bsal can spread by both active (e.g. salamanders, anurans) and passive (e.g. birds, water) carriers; it is most likely maintained/spread in infected areas by contacts of salamanders or by interactions with anurans, whereas human activities most likely cause Bsal entry into new areas and populations. In kept amphibians, Bsal contamination via live silent carriers (wild birds and anurans) is considered extremely unlikely. The risk-mitigation measures that were considered the most feasible and effective: (i) for ensuring safer international or intra-EU trade of live salamanders, are: ban or restrictions on salamander imports, hygiene procedures and good practice manuals; (ii) for protecting kept salamanders from Bsal, are: identification and treatment of positive collections; (iii) for on-site protection of wild salamanders, are: preventing translocation of wild amphibians and release/return to the wild of kept/temporarily housed wild salamanders, and setting up contact points/emergency teams for passive surveillance. Combining several risk-mitigation measures improve the overall effectiveness. It is recommended to: introduce a harmonised protocol for Bsal detection throughout the EU; improve data acquisition on salamander abundance and distribution; enhance passive surveillance activities; increase public and professionals' awareness; condition any movement of captive salamanders on Bsal known health status.

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Keywords: Bsal, salamanders, carriers, movements, wild and captivity, risk-mitigation measures

Requestor: European Commission

Question number: EFSA-Q-2017-00205

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Acknowledgements: The AHAW Panel wishes to acknowledge the following for the support provided to this scientific output: Tracey King and Paul Bakuwel (hearing experts), and Rhea Creve (visiting student from the University of Ghent-BE). The AHAW Panel wishes to acknowledge: F. Glaser, M. Grabher, P. Kaufmann, G. Ludwig, A. Maletzky, G. Munimanda, G. Pechlaner, D. Preininger, S. Smith, K. Smole-Wiener, S. Schweiger, T. Wampula, C. Walzer and volunteers. Department of Integrative Biology and Evolution, University of Veterinary Medicine Vienna and Schönbrunn Zoo (AU), F. Pasmans, Ghent University (BE), research funded by ANB (Flemish government), M. Lukac, Faculty of Veterinary Medicine, University of Zagreb (HR), D. Schmeller, UFZ (DE), R. Kostanjsek and N. Gunde Cimerman, Department of Biology, Biotechnical Faculty, University of Ljubljana (SL), J. Bosch, C. Monsalve and C. Sausor, CSIC (ES), A.A. Cunningham, research funded by the UK government (Defra, Welsh Government and the Animal and Plant Agency Diseases of Wildlife Scheme, Esmée Fairbairn Foundation and Universities Federation for Animal Welfare; UK) for providing information and data on the Bsal screening/surveillance activities in wild salamanders in some EU MSs for this scientific output.

Suggested citation: EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More S, Angel Miranda M, Bicout D, Bøtner A, Butterworth A, Calistri P, Depner K, Edwards S, Garin-Bastuji B, Good M, Michel V, Raj M, Saxmose Nielsen S, Sihvonen L, Spoolder H, Stegeman JA, Thulke H-H, Velarde A, Willeberg P, Winckler C, Baláz V, Martel A, Murray K, Fabris C, Munoz-Gajardo I, Gogin A, Verdonck F and Gortázar Schmidt C, 2018. Scientific Opinion on the risk of survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. EFSA Journal 2018;16(4):5259, 78 pp. <https://doi.org/10.2903/j.efsa.2018.5259>

ISSN: 1831-4732

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Summary

Batrachochytrium salamandrivorans (Bsal; Phylum Chytridiomycota, Order Rhizophydiales) is an emerging fungal pathogen of Caudata (salamanders and newts, from now on 'salamanders' in this scientific opinion) that was identified in 2013. Many European salamander species seem to be susceptible.

Upon request from the European Commission, in 2017, the European Food Safety Authority (EFSA) has produced: (i) a scientific and technical assistance on Bsal as a disease with the potential to harm kept and wild salamanders in the European Union (EU) with analysis of the various risk-mitigation options associated with imports of salamanders (and their products and by-products) into the EU and their trade within the EU; and (ii) the assessment of listing and categorisation of Bsal within the framework of the Animal Health Law.

The European Commission has also requested from EFSA a scientific opinion dealing with various aspects of the emerging pathogen, including environmental aspects, to understand the better possible scenarios for the evolution of this new disease, the current epidemiological situation, the experience gained so far from the implementation of the various control policies and possible alternative methods to diminish the negative effects on wild salamander populations and to ensure safe trade of kept animals and their products. Identification of gaps and uncertainties is also very important for this emerging disease.

Specifically, for this scientific opinion, EFSA was asked to assess: (a) as regards Bsal presence, absence, surveillance and eradication: (i) the possible identification of various areas (e.g. countries, zones, territories) which may be considered infected with Bsal or free from it; and (ii) the suitability of surveillance methods to ensure reliable and robust demonstration of the presence or absence of Bsal; (b) as regards spread of Bsal in and from infected areas or via infected animals or fomites: (i) the risk of survival, spread and establishment of Bsal within already infected areas and spread from infected areas into other parts of the EU by natural movements of live salamanders taking into account especially relevant geographical, hydrographical and meteorological conditions; (ii) the risk-mitigating measures that could potentially be effective in ensuring safer international or intra-EU trade of live salamanders (both captured in the wild and bred) and their products and by-products as regards the transmission of Bsal including diagnosis and potential treatment(s); and (iii) the role of live silent carriers of Bsal in spreading it as vectors and those of fomites (e.g. waste water, animal by-products, feed) and their risk-mitigating measures; and c) as regards the protection of salamanders from Bsal: (i) the potential and feasible risk-mitigating measures in kept salamanders and (ii) the risk-mitigating measures for wild salamanders in their natural habitats.

The situation of Bsal is currently very dynamic, and since the European Commission mandate was drafted several new cases and new knowledge has been reported. For instance, Bsal was found in wild salamander populations in Germany and in kept populations in Spain. In addition, in May 2017, the World Organisation for Animal Health (OIE) listed infection with Bsal in its Aquatic Animal Health Code, and the EU, with Commission Implementing Decision 2018/320, laid down certain animal health protection measures for import and intra-EU movements of salamanders in relation to Bsal that should apply temporarily (at least until 31 December 2019) until more information is available to supplement the current knowledge on Bsal.

This scientific opinion considers new information up to February 2018; methods included a combination of literature review, analysis of available information on: (i) Bsal detection; (ii) salamander abundance and distribution; (iii) how salamanders are transported and kept in captivity; and (iv) amphibian products and by-products, and expert assessment of risk-mitigation measures.

It was concluded that Bsal surveillance is currently limited. Despite this situation, Bsal has been detected in collections of captive salamanders in five EU Members States (MSs; Belgium, Germany, Spain, the Netherlands and the United Kingdom), and in wild populations in some regions of Belgium, Germany and the Netherlands. However, the absence of detection (in particular in the wild) does not mean the absence of Bsal. According to niche modelling, at least part of the range of distribution of every salamander species in the EU overlaps with the climate conditions predicted to be suitable for Bsal. However, the assumed distribution range of salamander species is based on a large spatial scale. Therefore, small-scale salamander habitat preferences may actually limit the total overlap between Bsal and salamanders. Bsal-specific real-time polymerase chain reaction (PCR) (qPCR) has been shown to have a high specificity and sensitivity, being a reliable and feasible diagnostic tool, and passive surveillance is considered the most suitable approach for detection of Bsal emergence. Demonstration of Bsal absence in wild populations of salamanders is currently considered not feasible, whereas it

would be feasible to demonstrate the absence of Bsal in a 'closed population' of a susceptible salamander species kept in captivity by sufficiently long quarantine and the absence of clinical symptoms of Bsal confirmed by visual observation. This should be complemented by testing all animals at the end of a quarantine period.

In the wild, Bsal can possibly be spread by both active carriers (e.g. salamanders, anurans) and passive carriers (e.g. wild birds, water). Bsal is most likely maintained and/or spread in infected areas by intraspecies and interspecies contacts of salamanders but potentially also by interactions with anurans. Bsal entry into new areas and populations is most likely to be caused by human activities (mainly amphibian-related), but potentially also by wild birds, wild mammals and through connected streams of water. For on-site protection of wild salamanders in their natural habitats, the risk-mitigating measures that were considered the most feasible and effective ones are: preventing release/return to the wild of kept or temporarily housed wild salamanders, preventing the translocation of wild amphibians, and setting up contact points/emergency teams in support of passive surveillance. Mitigation measures on hygiene procedures were considered feasible for anthropogenic sources of risk, but less effective for natural mechanisms of spread (e.g. intraspecies or interspecies transmission, potential carriers, such as wild birds). The effectiveness is likely to increase when combining several mitigation measures as part of an integrated approach.

The risk-mitigation measures that were considered most feasible and effective in ensuring safer international or intra-EU trade of live salamanders are: either a ban or restrictions on salamander imports, hygiene procedures and good practice manuals. The feasibility and effectiveness of a ban or restriction on salamander import and trade depend on the volumes of animals that are currently transported; these measures might also boost illegal movements. The effectiveness of heat treatment was also considered high. However, the heat tolerance of many salamander species is unknown. Combining several risk-mitigation measures (in an integrated infection management strategy) improves overall effectiveness. No information is available on import of salamander products and by-products, however they are subjected to treatments (i.e. heat-processing or desiccation) that are considered feasible and effective to ensure safe international and intra-EU trade.

In populations of kept amphibians, Bsal spread can potentially occur also via passive carriers such as human movements and activities, waste water, equipment, substrate and fomites. These risks can be mitigated by implementing hygiene procedures and good practices. Bsal contamination of traded and/or kept salamanders via live silent carriers (wild birds and anurans) is considered extremely unlikely, as, if good practices are implemented, salamanders are normally kept indoors and separated from anurans. The risk-mitigation measures that were considered the most feasible and effective ones for protection from Bsal of kept salamanders in captivity are: the identification and treatment of positive collections. However, also increasing stakeholders' awareness (salamander breeders, keepers, pet-shops and stores), good practice and hygiene protocols were considered feasible and their effectiveness can be increased by combining the available measures into an integrated infection management strategy.

Recommendations derived from these findings include: (i) introducing a harmonised protocol for Bsal detection throughout the EU; (ii) improving data acquisition on abundance and distribution of salamanders as well as enhancing passive surveillance activities for Bsal; (iii) increasing awareness among professionals and the public; (iv) conditioning any movement of captive animals on known health status (Bsal test negativity); and (v) distributing guidelines on hygiene procedures and best practices to all relevant stakeholders and foreseeing that they become integral components of permits for dealing with wild salamanders (site visitation, capture, handling and movement).

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Batrachochytrium salamandrivorans (Bsal) was identified by scientists as recently as in 2013. Over the last couple of years Bsal has occurred at least in certain parts of Europe either in the wild population of salamanders and newts (e.g. the Netherlands, Belgium in several locations) or in kept population (e.g. Germany, UK) or possibly in both populations. There are no data from other EU countries but similar cases either in wild or in kept salamanders cannot be excluded. In certain areas (e.g. the Netherlands) this fungus is said to have devastated local fire salamander populations. In other places the fungus is apparently present in susceptible species, but without increased mortalities. Many Asian salamander species seem to be immune or tolerant to this pathogen to various extents. Many European species seem to be susceptible.

This situation and state of knowledge is patchy, fragmented and is expected to change as more knowledge about this emerging pathogen and especially surveillance data become available, continually and gradually. Overall, scientific data on Bsal are still scarce with significant gaps. Currently the disease is not listed under OIE standards or in the EU rules.

A few affected countries, or those that anticipate that Bsal could affect them, have adopted diverse control policies or are considering various possible measures against the disease to cope with its feared short-term and long-term consequences in wild animals and in kept salamanders and their trade. One such example is an import ban of certain salamander species introduced by the USA, where Bsal is either absent or not yet detected. Other measures are of a non-legislative nature, such as raising awareness among stakeholders on risks, guidelines for improved biosecurity or survey salamander populations, and any changes, with the emphasis on increased mortalities and/or occurrence of Bsal. To date, these have been carried out under environmental policies.

Some individuals have recently called for inter alia, EU animal health policy and legislative measures to be adopted, in particular an immediate ban on the import of many species of salamanders from Asia into the EU. It has been shown by phylogenetic analysis that the fungus indeed originates in certain parts of Asia. Therefore, it has been speculated by some that trade in Asian salamanders may have played a role in its spread into and within the EU, although there is no proof that this pathogen entered the EU via this route and, if so, when and under what circumstances. In general, details are missing on its spread into or in the EU or between kept and wild animals.

The Commission therefore needs a quick but comprehensive compilation, scrutiny of available data and assessment to determine if Bsal is a disease with the potential to harm kept and wild salamanders in the Union and various risk factors associated with:

- imports of Asian salamanders into the EU and their trade within the EU;
- movements of European salamanders (both caught from the wild or kept ones) within the EU, and imports and movements of animal by-products obtained from Asian and European salamanders (both caught from the wild or kept ones).

Such assessment would be essential for the consideration of potential safeguard measures in relation to imports from Asia or for movements from infected to non-infected EU areas.

In the past, EFSA has produced scientific opinions dealing with various aspects of emerging pathogens, including those in which environmental aspects or wild animals play an increased role or are affected (such as, for example, on small hive beetle). Therefore, a similar opinion is necessary to understand better possible scenarios for the evolution of this new disease, the current epidemiological situation, the experience gained so far from the implementation of the various control policies and possible alternative methods to diminish negative effects on wild salamander populations and to ensure safe trade of kept animals and their products. Identification of gaps and uncertainties is also very important for this emerging disease.

Furthermore, EFSA has already been made aware of the adoption and publication of the Regulation on transmissible animal diseases (Animal Health Law, AHL). As Bsal is not included in the list of diseases in Annex II to the AHL (or on the list of any other existing EU animal health legislation), environmental players have asked the Commission to place Bsal onto that list. Therefore, a review of this list will be necessary under a set of criteria provided for in the AHL before it comes into force, taking into account the transitional periods envisaged for its application (five years starting from April 2016). Hence the Commission needs scientific advice for the assessment of the significance of Bsal

within the framework of this already known listing and categorisation according to the AHL, in the same manner as was requested previously for another two groups of diseases [Ref. SANTE G2/BL/lp (2015) 4940871, SANTE G3/LPA/lp (2016) 3154863, respectively].

The criteria, provided for in Articles 7 and 8 and Annex IV of the AHL shall be used as a basis for this analytical assessment. The risk manager needs a scientific advice to:

- 1) assess if Bsal causes disease for which control measures at the EU level are justified;
- 2) proceed with the profiling of the disease in view to its categorisation;
- 3) assign listed species to Bsal identified as eligible for EU intervention.

The Commission have identified the main issues for which concrete elements of science may provide good basis for formulating policies and/or adapt current approach. These are as follows:

- provisions for safe trade (entry into the Union and trade within the Union) with Asian and European salamanders and animal by-products obtained therefrom;
- identifying links between groups of salamanders in trade (i.e. in consignments being moved or in shops, etc.) and kept ones (i.e. stationary, whether for hobby or else) and salamanders in wild (i.e. in their natural habitat) and possible routes and risks of spreading Bsal between the specimens belonging to the above three groups and locations;
- effects of the respective infection of salamanders with Bsal, including aspects stemming from different susceptibility of various species to Bsal;
- measures to monitor occurrence of Bsal in those groups and mitigate mortality due to Bsal, whether regulatory measures or non-regulatory ones.

1.1.1. Terms of Reference

I. Scientific and technical assistance in accordance with Article 31 of Regulation (EC) No 178/2002

In view of the above, in accordance with Article 31 of Regulation (EC) No 178/2002, the Commission asks EFSA to provide scientific and technical assistance concerning:

- 1) assessment of the potential of Bsal to affect the health of wild and kept salamanders in the Union;
- 2) effectiveness and feasibility of a movement (including intra-EU trade and introduction from non-EU countries) ban of traded salamanders, including both Asian and non-Asian species;
- 3) the validity, reliability and robustness of the available diagnostic methods for the detection of Bsal;
- 4) possible alternative methods and feasible risk-mitigation measures to ensure the safe international and EU trade of salamanders and their products.

II. Scientific opinion in accordance with Article 29 of Regulation (EC) No 178/2002

In accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA to provide a scientific opinion on the following:

1) As regards susceptibility, morbidity and mortality, assess (EFSA, 2017):

- a) the susceptibility and morbidity of various Asian and European salamanders to Bsal (also EFSA AHAW Panel, 2017);
- b) the nature of Bsal as facultative or not pathogen of European salamanders;
- c) if there are species of salamanders carrying Bsal without clinical symptoms and/or clinical and serological evidence and if so, which ones;
- d) the mortality rates of native European salamander species due to Bsal (also EFSA AHAW Panel, 2017);
- e) the role of other factors (e.g. habitat degradation) in increased mortalities associated with Bsal.

2) As regards presence, absence, surveillance and eradication, assess:

- a) the risk of survival and establishment of Bsal in the environment in the EU under various meteorological conditions (EFSA, 2017);
- b) possible identification of various areas (e.g. countries, zones, territories) that may be considered infected with Bsal or free from it (this scientific opinion);

- c) the definition of requirements for reliable detection of Bsal in the wild in affected areas or exclusion of its presence (EFSA, 2017; EFSA AHAW Panel, 2017);
- d) the suitability of surveillance methods to ensure the reliable and robust demonstration of the presence or absence of Bsal (this scientific opinion).

3) As regards spread of Bsal in and from infected areas or via infected animals or fomites, assess:

- a) the risk of survival, spread and establishment of Bsal within already infected areas and spread from infected areas into other parts of the EU under various scenarios:
 - i) by natural movements of live salamanders taking into account especially relevant geographical, hydrographical and meteorological conditions (this scientific opinion);
 - ii) by movements of traded live salamanders and their traded products, body parts, etc. from infected areas, both under identified risk-mitigation measures or without (EFSA, 2017);
- b) risk-mitigating factors that could potentially be effective in ensuring safe international or intra-EU trade of live salamanders (both captured in the wild and bred) and their products and by-products as regards the transmission of Bsal including diagnosis and potential treatment(s) (this scientific opinion);
- c) the role of live silent carriers of Bsal in spreading it as vectors and those of fomites (e.g. waste water, animal by-products, feed) and their risk-mitigating measures (this scientific opinion);
- d) the possible routes of spread between kept salamanders, originating from international trade and the autochthonous salamanders living in the wild, i.e. their natural habitat (EFSA, 2017; EFSA AHAW Panel, 2017).

4) As regards on-site protection from Bsal, assess (this scientific opinion):

- a) the potential and feasible risk-mitigating factors and methods in kept salamanders;
- b) the risk-mitigating factors and methods for salamanders in their natural habitat.

5) Listing and categorisation of Bsal in the framework of the Animal Health Law (EFSA AHAW Panel, 2017):

- a) assess, following the criteria laid down in Article 7 of the AHL, its eligibility of being listed for Union intervention as laid down in Article 5(3) of the AHL;
- b) if found eligible to be listed for Union intervention, provide:
 - i) an assessment of its compliance with each of the criteria in Annex IV to the AHL for the purpose of categorisation of diseases in accordance with Article 9 of the AHL;
 - ii) a list of animal species that should be considered candidates for listing in accordance with Article 8 of the AHL.

1.2. Interpretation of the Terms of Reference

Batrachochytrium salamandrivorans (Bsal; Phylum Chytridiomycota, Order Rhizophydiales) was first described in 2013 (Martel et al., 2013) and is the pathogen of lethal chytridiomycosis in salamanders. For taxonomic consistency, in this document the use of the term 'salamanders' is used as synonymous of 'Caudata' and is inclusive of 'newts'.

The situation of Bsal is currently very dynamic and since the mandate was drafted several new cases and new knowledge occurred. For instance Bsal was found in wild salamander populations in Germany and in kept populations in Spain. In addition, in May 2017, the World Organisation for Animal Health (OIE) listed infection with Bsal in its Aquatic Animal Health Code (OIE, 2017), and the European Union (EU), with Commission Implementing Decision 2018/320¹, laid down certain animal health protection measures for import and intra-EU movements of salamanders in relation to Bsal that should apply temporarily (at least until 31 December 2019) until more information will become available to supplement current knowledge on Bsal.

This scientific opinion considers new information up to February 2018.

¹ Commission Implementing Decision (EU) 2018/320 of 28 February 2018 on certain animal health protection measures for intra-Union trade in salamanders and the introduction into the Union of such animals in relation to the fungus *Batrachochytrium salamandrivorans*. Official Journal L 62, 5.3.2018, pp. 18–33.

The four Terms of Reference (ToRs) relevant to the scientific and technical assistance in accordance with Article 31 of Regulation (EC) No 178/2002² (see point I above) have already been addressed by EFSA with the scientific report published at the end of February 2017 (EFSA, 2017). The same report has also addressed ToR 1, and some aspects of ToR 2 (points a and c) and of ToR 3 (points a–ii and d) of the ToRs relevant to the scientific opinion in accordance with Article 29 of Regulation (EC) No 178/2002 (point II above).

A separate scientific opinion (EFSA AHAW Panel, 2017) has addressed ToR 5 and some aspects of the other ToRs (i.e. ToR 1 points a and d; ToR 3 point d) of the ToRs relevant to the scientific opinion in accordance with Article 29 of Regulation (EC) No 178/2002 (point II above).

Therefore, as specified in parentheses at the end of each ToR, this scientific opinion aims at addressing the following ToRs relevant to the scientific opinion in accordance with Article 29 of Regulation (EC) No 178/2002 (point II above): points b and d of ToR 2; points a–i, b and c of ToR 3; and ToR 4.

For ToR 2b and d, the assessment will focus on the demonstration of the presence of Bsal. An ecological model of Bsal presence leading to opportunity maps in the EU based on the currently recorded findings on Bsal will be described; data from activities of Bsal early detection and active and passive surveillance carried out in some Members States (MSs) will also be discussed. Considerations on the difficulties of assessing and confirming Bsal absence in the wild will also be provided.

Spread is the process of active or passive movement of the pathogen from one suitable area/population to another area/population in which establishment can occur. For addressing ToR 3a–i, the possible means of Bsal spread (carriers) in the salamanders' natural habitats will be identified. It is differentiated to 'active carriers', which are hosts acting like biological vectors for the spores, and 'passive carriers', which are similar to mechanical vectors to which the spores can be attached. Both categories of carriers will then be categorised by their relevance for spread of Bsal in wild live salamanders, in three scenarios: (i) within a subpopulation, (ii) from an infected area to an uninfected area between subpopulations of the same meta-population, and (iii) from an infected area to an uninfected area across geographical barriers (between meta-populations).

For consistency with the terminology used in other EFSA's scientific outputs, for ToRs 3b, 4a and 4b of this opinion, the assessment will refer to risk-mitigation 'measures' and not to 'factors'.

For ToR 3b, the risk-mitigating measures that could potentially be effective in ensuring safer international or intra-EU trade of live salamanders and reducing the risks of Bsal, will be described; a qualitative assessment of the related feasibility and effectiveness will be performed also considering the consequences. Considerations on possible salamander products and by-products as regards the transmission of Bsal will be also provided. According to the wording of ToR 3c, the Bsal-silent carrier concept refers to the Bsal-passive carriers. Information of these carriers of Bsal, their products and by-products will be provided together with the relevant risk-mitigation measures.

'On-site' protection conventionally refers to wild animals in their natural habitats; in this opinion the assessment of ToR 4 will refer to the protection of captive salamanders in places where they are kept and the 'on-site' protection of wild salamanders in their natural habitats (ToR 4b). Measures that can be put in place for their protection will be described and assessed for effectiveness and feasibility.

When no evidence on Bsal was available, but studies on the sister species *Batrachochytrium dendrobatidis* (Bd) were available, reference to these studies on Bd has been reported in this scientific opinion because the two fungi have been shown to be close enough to allow making some generalisations on basic biological aspects for both species of the genus *Batrachochytrium* (for more details, see EFSA, 2017).

2. Data and methodologies

2.1. Data

2.1.1. Data from the literature

Information from the papers selected as relevant from the Extensive Literature Search (ELS) described in Section 2.2.1, and from additional literature identified by the experts was used for a narrative description and assessment to address ToRs 2, 3 and 4; the results from a previous ELS

² Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal L 31, 1.2.2002, pp. 1–24.

carried out in 2016 for the EFSA Scientific Report on Bsal (EFSA, 2017) were also taken into consideration for the assessment in this scientific opinion.

2.1.2. Salamander population data

The abundance of salamanders in the EU is still not comprehensively known.

Continuous population monitoring of the species in Annex II of the Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora the Habitats Directive should be performed by all the EU MSs.³

The salamander species of community⁴ interest (Annex II of the Council Directive 92/43/EEC) are: *Chioglossa lusitanica*, *Lyciasalamandra* (synonym (syn.). *Mertensiella*) *luschani*, *Salamandra aurorae* (*Salamandra atra aurorae*), *Salamandrina terdigitata*, *Triturus carnifex*, *Triturus cristatus*, *Triturus dobrogicus*, *Triturus karelinii*, *Lissotriton* (syn. *Triturus*) *montandoni*, *Lissotriton* (syn. *Triturus*) *vulgaris ampelensis*, *Proteus anguinus*, *Speleomantes ambrosii*, *Speleomantes flavus*, *Speleomantes genei*, *Speleomantes imperialis*, *Speleomantes strinatii* and *Speleomantes supramontis*. Data on the monitoring results are publicly available online.⁵

Fragmented activities at EU level on collection of data on the size and distribution of salamander population have been reported in the previous EFSA Scientific report (EFSA, 2017).

An inventory of all monitoring events in the different MSs requires extensive study, e.g. a survey with all relevant stakeholders (governments, non-governmental organisations (NGOs), research and academic institutions); this information is highly fragmented and not available in a central database. Monitoring efforts are often project related in a defined area or for some species. For example, Speybroeck and Steenhoudt (2017) provided the example of an available database on *Salamandra salamandra* with population relevant information.

On the basis of the experts' knowledge, it was possible to identify some MSs that have put in place systems for salamanders' population monitoring and species distribution mapping.

Some countries (e.g. the Netherlands) have systematic, country-wide monitoring systems in place (e.g. RAVON⁶). Some examples of taxon-specific monitoring programmes are: *Salamandra salamandra* and *Triturus cristatus* monitoring in Flanders (Belgium) and the Netherlands, *Salamandrina perspicillata* monitoring in Latium (Italy), amphibian monitoring in Sierra Norte de Sevilla (Spain), amphibian monitoring in Białowieża National park (Poland), and amphibian monitoring in Millevaches regional natural park (France).

Another example is the situation in the Czech Republic, where it is organised by the National Conservation Agency and includes four salamander species: *Triturus cristatus*, *Triturus carnifex*, *Triturus dobrogicus* and *Lissotriton montandoni*. The data collected on the species are submitted to the European Commission in 6-year cycles. Furthermore, long-term monitoring of selected species (including all four before-mentioned Caudata) in a representative network of sites has been carried out continuously since 2006, with the aim of detecting trends in populations. For *Triturus cristatus*, 30 permanently monitored sites are distributed in a regular fashion across the country. Each site is visited at least three times in the season: two visits in time of reproduction and one in the time of larval metamorphosis. Three options of quantification of population size are used – capture success of larvae and adults, visual counting of adults and live trapping success. Additional data on habitat quality and changes are collected along with the salamander monitoring.

The International Union for Conservation of Nature (IUCN) Red List⁷ database also provides data on the salamanders' species distribution in the EU.

2.1.3. Data from Bsal surveillance systems

A Bsal surveillance programme is not currently harmonised across the MSs.

On the basis of the experts' knowledge, it was possible to identify which MSs have put in place passive or/and active Bsal surveillance systems.

³ <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:01992L0043-20130701>

⁴ It is a formal term, for referring to (in full): 'Annex II of the Council Directive 92/43/EEC: animal and plant species of community interest whose conservation requires the designation of special areas of conservation'. <http://eunis.eea.europa.eu/references/2325/species>

⁵ http://ec.europa.eu/environment/nature/knowledge/rep_habitats/index_en.htm

⁶ Reptile, Amphibian and Fish Conservation the Netherlands (RAVON), <https://www.gbif.org/publisher/8629d002-c1cf-4c61-9895-55ca7cd0e149>

⁷ <http://www.iucnredlist.org>

Data on active and passive surveillance have been collected for *Salamandra salamandra* and other salamander species in some sites outside Prague in the Czech Republic, and for different species in some regions of other countries, such as: Austria, Belgium, Croatia, France, Germany, Portugal, Spain, Slovenia, Switzerland, the Netherlands and the UK. The surveillance has been non-systematic in the MSs and was carried out on an ad hoc basis (for more details, see Section 2.2.2.2).

Results of the surveillance activities are reported in Section 3.1.1.3 and Appendix B.

The relevant data have been used to assess the surveillance approach to detect the presence of Bsal and for feeding the discussion on demonstration of absence of Bsal (freedom from disease).

2.2. Methodologies

2.2.1. Extensive literature search

The previous ELS carried out in 2016 (EFSA, 2017) was updated by retrieving any peer-reviewed and grey literature containing information on Bsal until October 2017. Full details of the search protocol and strategy are reported in Appendix A.

The search yielded a total of 957 records. After de-duplication, the search resulted in 250 records. A first screening of all titles and abstracts was performed in parallel by two assessors to remove additional duplicates (if any) and to identify the literature pertaining to Bsal or pertaining to related aspects clearly relevant for Bsal, leading to 47 records (43 relevant papers plus 4 supplementary materials). Full-text screening reduced the number of relevant papers to 26 (see Tables A.1 and A.2). The identified papers were used to underpin the different ToRs.

The reference list of relevant review articles and key reports were checked for further relevant articles and experts were invited to propose any additional relevant publications.

2.2.2. The approach to surveillance – Demonstration of Bsal presence, absence, surveillance and eradication of Bsal (ToR 2)

2.2.2.1. Bsal niche modelling

Identification of hosts or regions susceptible to colonisation by an emerging pathogen can be achieved overlaying host distribution maps with regional mapping of Bsal suitable climate.

2.2.2.2. The climatic niche of Bsal

The climatic niche of Bsal has been modelled based on temperature and precipitation data of sites of known occurrence of Bsal in its native Asian range and its invasive European distribution. The model was recently described (Beukema et al., in preparation).⁸ The potential occurrence of Bsal was then assessed for its overlap with the climatic niches of 56 putative Western Palearctic host species. Such analyses may provide support for directed conservation actions, including mitigation prioritisation. However, modelling the niche of a species (the range of climates where it is, or could be, present) is complex, particularly, for invasive species. Typically, such correlative models for invasive species utilise occurrence records in the species' native area to estimate the species' response to a range of environmental factors that together define a species distribution (e.g. temperature or rainfall). However, when moving into an invaded range, it cannot be guaranteed that the range of biotic and abiotic conditions that were present in the native range also occur in the invaded range. For example, a new combination of climatic factors might occur in the invaded range that have not been encountered in the native range, resulting in model extrapolation beyond the data used to train the model in the invaded region, which could affect model validity and accuracy. Similarly, 'hidden' components of a species niche that would be implicitly captured in models based on a native range may be missing in the invaded range. This situation is particularly important for a pathogen, as the characteristics of host–pathogen dynamics are often strongly mediated by hosts (as per Bd). As such, care must be taken in evaluating the assumptions of building models from one host–pathogen system (i.e. in native range) and projecting into another (i.e. invaded range).

⁸ The AHAW Panel had the opportunity to review the manuscript: Beukema W, Martel A, Nguyen TT, Goka K, Schmeller DS, Yuan Z, Laking AE, Nguyen TQ, Lin CF, Sheldon J, Loyau A and Pasmans F. Environmental context and changes in niche structure influence estimating *Batrachochytrium salamandrivorans* invasion risk in the Western Palearctic. In preparation.

2.2.2.3. Bsal surveillance systems in Europe

In the Czech Republic, active surveillance of Bsal was carried out within Prague's urban area and sites in its vicinity. This activity started in 2015, in several sites considering the possibility of the pathogen to spill over from kept salamanders to the wild. Samples of captive salamanders were available for Bsal analyses from previous research projects (Havlíková et al., 2015) and by collaboration with zoos and private breeders. The sampling sites had a good accessibility to the live salamander populations, which were tested by swabbing the skin; initially 126 samples from nine sites were collected in autumn 2015 and spring 2016 (Baláz et al., 2018). Sampling continued and altogether 12 sites were visited in search of *Salamandra salamandra*. The first visit on each site was performed to identify the exact microhabitats of salamander occurrence and the possibility of sampling, taking around 3–4 person hours per site. The second visit consisted of sampling, two people spending 3–6 h on site locating and sampling salamanders. Pathogen diagnostic was performed by duplex real-time polymerase chain reaction (qPCR) (Blooi et al., 2013). Although the surveys were originally planned to be repeated each year, in 2017, a shortage of manpower and limited funding ceased the activities (Baláz et al., 2018).

Apart from the Directive 92/43/EEC⁹ on species' monitoring (mentioned in Section 2.1.2), in the Czech Republic a fine-scale mapping scheme of all amphibians started in 2008 under the lead of the National Conservation Agency (NCA CR) and since 2014 it covers the full extent of the country. The people involved in the monitoring and mapping are informed about the need to collect and report dead and sick animals to the Agency, which then contacts the researchers involved in chytridiomycosis research for sample analyses.

To identify other countries where Bsal surveillance systems have been put in place, a questionnaire was circulated by contacting research institutes, diagnostic laboratories and NGOs from Austria, Croatia, France, Germany, Italy, Slovenia, Spain, Sweden, Switzerland, the Netherlands and the UK to provide currently available data on Bsal investigations in wild amphibians in Europe.

Passive surveillance, using 'disease emergency teams', is in place in Belgium, France, Germany, Italy, Spain, the Netherlands and the UK.¹⁰ All known Bsal outbreaks in Belgium, Germany and the Netherlands were detected using passive surveillance. Funding for these teams (in the above-mentioned countries) is provided by the European Commission until 2020. In Belgium, funding has been provided by the government for yearly active surveillance of 10 sentinel populations of fire salamanders in Flanders from 2015 until 2018 and for screening of 20 fire salamander populations in Wallonia in 2016 and 2017.

In Austria, Belgium, Croatia, Czech Republic, France, Germany, Portugal, Slovenia, Spain, Switzerland, the Netherlands and the UK, non-systematic active surveillance has been carried out on an ad hoc basis.

For an overview of the surveillance systems currently in place in Europe and their funding, see Table 1 below.

Table 1: Duration and funding of the active Bsal surveillance programmes place in some European countries

Country	Start	End	Funding
Austria	2016	2017	Mainly private, small part funded by state and research fund
Belgium	2015	2018	Government, research fund, European Commission
Croatia	2017	2018	Research fund
Czech Republic	2015	2016	University research funding
France	2017	2018	European Commission
Germany	2016	2018	Government, research fund, European Commission
Portugal	2015	2015	Research fund
Slovenia	2017	2017	Government, Slovenian research agency
Spain	2016	2017	Research fund, European Commission
Switzerland	2008	2013	Research fund
Netherlands	2013	2018	Government
United Kingdom	2014	2017	Government, European Commission, NGO, private foundation

⁹ Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. OJ L 206, 22 July 1992, pp. 7–50.

¹⁰ For more information: <https://bsalinforeurope.wixsite.com/eubsalmitigation2017>

2.2.3. Risk of survival, spread and establishment of Bsal within already infected areas and spread from infected areas into other parts of the EU by natural movements of wild live salamanders taking into account especially relevant geographical, hydrographical and meteorological conditions (ToR 3a-i)

The Working Group (WG) experts were asked to produce from the available literature and their own knowledge a list containing the possible means of Bsal spread into the salamanders' natural habitat together with their description and relevant geographical, hydrographical and meteorological considerations.

A consensus discussion addressed the means for the role in Bsal spread and entry. All means were ranked by their relevance for spread of Bsal in wild live salamanders and put in three different categories dependent on the following scenarios of spread: (i) within a subpopulation, (ii) from an infected area to an uninfected area between subpopulations of the same meta-population, and (iii) from an infected area to an uninfected area across geographical barriers (between meta-populations), using categories from 'A' to 'C'. 'A' represents means that were categorised as most relevant for Bsal spread in a certain scenario, whereas 'B' and 'C' were categorised as of lower importance. If necessary, subcategories were introduced to express within category differences using small letters: a, b and c (see Table 2).

2.2.4. The approach to ToRs 3b, 4a, 4b

To address the following ToRs, the WG experts were asked to produce three lists from the available literature and their own knowledge.

For ToR 3b on the transmission of Bsal, the main risk-mitigating measures that could be potentially effective in ensuring safer international or intra-EU trade of live salamanders, their products and by-products. The list of potential measures for Bsal management proposed by Grant et al. (2016) was already assessed for relevance in EFSA (2017). For this scientific opinion, the experts were asked to revise that list, and, on the basis of the new available knowledge, to add any additional mitigation options that could be considered relevant and to assess them for feasibility and effectiveness.

For ToRs 4a and 4b, the risk-mitigating measures and methods for protection of salamanders where they are kept in captivity and of populations of wild salamanders in their natural habitats (on-site), respectively.

2.2.4.1. The assessment of feasibility

Feasibility of the identified measures was qualitatively assessed by the experts for ToRs 3b, 4a and 4b.

For ToR 3b, the feasibility of each of the risk-mitigating measures that were considered to be potentially effective in ensuring safer international or intra-EU trade of live salamanders was assessed on a continuous scale from 'least feasible' to 'most feasible', integrating aspects related to human resources, technical efforts, treatment costs and environmental side-effects.

'Least feasible' means that the implementation of the measure demands extensive resources, e.g. it requires lots of personnel (qualified personnel handling most of the individuals) and a large amount of money (e.g. expensive reagents and facilities; complexity of monitoring the conduct), or if it is dangerous to other species or the environment (e.g. causing toxicity – or resistance).

'Most feasible' means that the measure's implementation demands few resources for personnel (e.g. it does not require additional qualified personnel), amount of money (e.g. cheap reagents and existing facilities/does not require reagents and facilities) and implies negligible danger to other species or the environment.

For ToRs 4a and b, the feasibility of each of the risk-mitigating measures and methods that were considered relevant for the protection of kept salamanders and for 'on-site' protection of populations of wild salamanders in their natural habitats was assessed on a continuous scale from 'least feasible' to 'most feasible', integrating aspects related to human resources, stakeholder willingness to comply/ implement the measure, technical complexity, feasibility of monitoring the implementation, treatment costs and long-term outlook, e.g. permanent efforts.

'Least feasible' is defined as the implementation demanding extensive resources, e.g. when it requires lots of personnel, or only few stakeholders will comply, when the implementation and conduct of the procedures are complicated, if the resource needs are high, or requires new equipment or facilities to be build/bought, and when a long-term outlook requires constantly high-level efforts.

'Most feasible' means that the measure's implementation is straight forward, demanding few resources (e.g. it requires few people per hour, no additionally qualified personnel is needed), most of the stakeholders will comply, it requires inexpensive equipment and already existing facilities, when the implementation and conduct procedures are simple, and a long-term application is easily sustainable.

For all the three ToRs, after consensus appraisal of the outcomes of the individual judgements (see details in Appendix C, Sections C.3.1, C.4.1 and C.5.1) and discussion of particular reasoning, the overall outcome was represented by an interval covered by the central estimates across all experts. The quintiles of value distribution of judgements were converted into a scale from 1 to 5 (see Tables 3, 4 and 5). The broader is the reported interval, the greater is the uncertainty comprised in the judgements.

2.2.4.2. The assessment of effectiveness

Effectiveness of the identified measures was qualitatively assessed by the experts for ToRs 3b, 4a and 4b.

For ToR 3b, the effectiveness to prevent translocation of Bsal through any means of each risk-mitigating measure was assessed on a continuous scale from 0 – ‘no prevention’ to 1 – ‘blocking measure’.

For ToRs 4a and 4b, the effectiveness of each measure to protect kept/wild salamanders against Bsal infection was assessed on a continuous scale from 0 – ‘negligible’ to 1 – ‘fully protective’.

‘Negligible’ for kept salamanders means that the activity does not alter the exposure to Bsal introduction to uninfected populations or the further perpetuation between salamanders within an infected population; for wild salamanders, it means that the activity does not alter the exposure to Bsal introduction of an uninfected site or the further perpetuation between salamanders on an infected site.

‘Fully protective’ means that the activity leads to refractory protection of uninfected salamanders in a kept population against Bsal infection or that the activity leads to refractory protection of uninfected wild salamanders on a site against Bsal infection (for on-site protection).

For all the three ToRs, after consensus appraisal of the outcomes of the individual judgements (see Appendix C, Sections C.3.1, C.4.1 and C.5.1) and discussion of particular reasoning, the overall outcome was represented by an interval covered by the central estimates across all experts. The quintiles of value distribution of judgements were converted into a scale from 1 to 5 (see Tables 3, 4 and 5). The broader is the reported interval, the greater is the uncertainty comprised in the judgements.

2.2.4.3. The assessment of uncertainty

In the experts’ judgement for ToRs 3b, 4a and 4b, a particular value was elicited and according to EFSA’s Guidance (2014) the uncertainty of each judgement was quantified. Experts provided a minimum and maximum to frame the elicitation and then the central value and the interquartile range of the distribution were elicited (for details see Appendix C, Section C.2).

2.2.5. Identification of amphibian products and by-products (ToR 3b and c)

Possible amphibian products and by-products have been identified on the basis of the experts’ knowledge, information gathered from the ELS from a first search that was conducted using different key words in Google such as: ‘amphibian products’, ‘amphibian derived products’, ‘animal by-products list’, ‘products made from frogs’, ‘frog products’, ‘frog by-products’.

Based on the results more specific key words were used in web search engines (google, Web of Science, European Commission website), such as: ‘Chinese giant salamander’, ‘salamander meat’, ‘frogs’ legs EU’, ‘frogs’ legs import EU’, ‘frogs’ legs frog species’, ‘EU trade of animal products’.

The potential of the identified amphibian products and by-products to pose a threat to EU salamanders was then discussed by the experts (see Sections 3.2.3.1 and 3.2.4.6).

3. Assessment

3.1. Bsal presence, absence, surveillance and eradication (ToR 2)

3.1.1. Possible identification of various areas (e.g. countries, zones, territories) which may be considered infected with Bsal or free from it (ToR 2b)

3.1.1.1. Considerations on Caudata diversity and distribution in the EU

On the basis of the most recent scientific knowledge, the order Caudata is currently composed of 10 families divided into 68 genera and 710 species (from Amphibiaweb¹¹). The taxonomy, distribution and abundance, conservation status and trends of salamanders’ population have been described in

¹¹ <https://amphibiaweb.org> University of California, Berkeley, CA, USA. Accessed 11 January 2018.

details in a previous EFSA's scientific report (EFSA, 2017). Compared with the previous EFSA publication, a new family of salamander has been recognised in northern America: Dicamptodontidae, which contains four species. This factor is only of taxonomical importance and it has no effect on the situation in the EU.

EU MSs host a considerable salamander species diversity: 37 species are recorded to be present in the wild (Temple and Cox, 2009; Sillero et al., 2014) and they pertain to three families: Plethodontidae, Proteidae and Salamandridae (see also Table C.2 of EFSA, 2017).

Data on distribution of salamander species are available; however, abundance data are generally not comprehensive.

The IUCN Red List contains data for 35 species present in the EU and Figure 1 shows their spatial distribution.

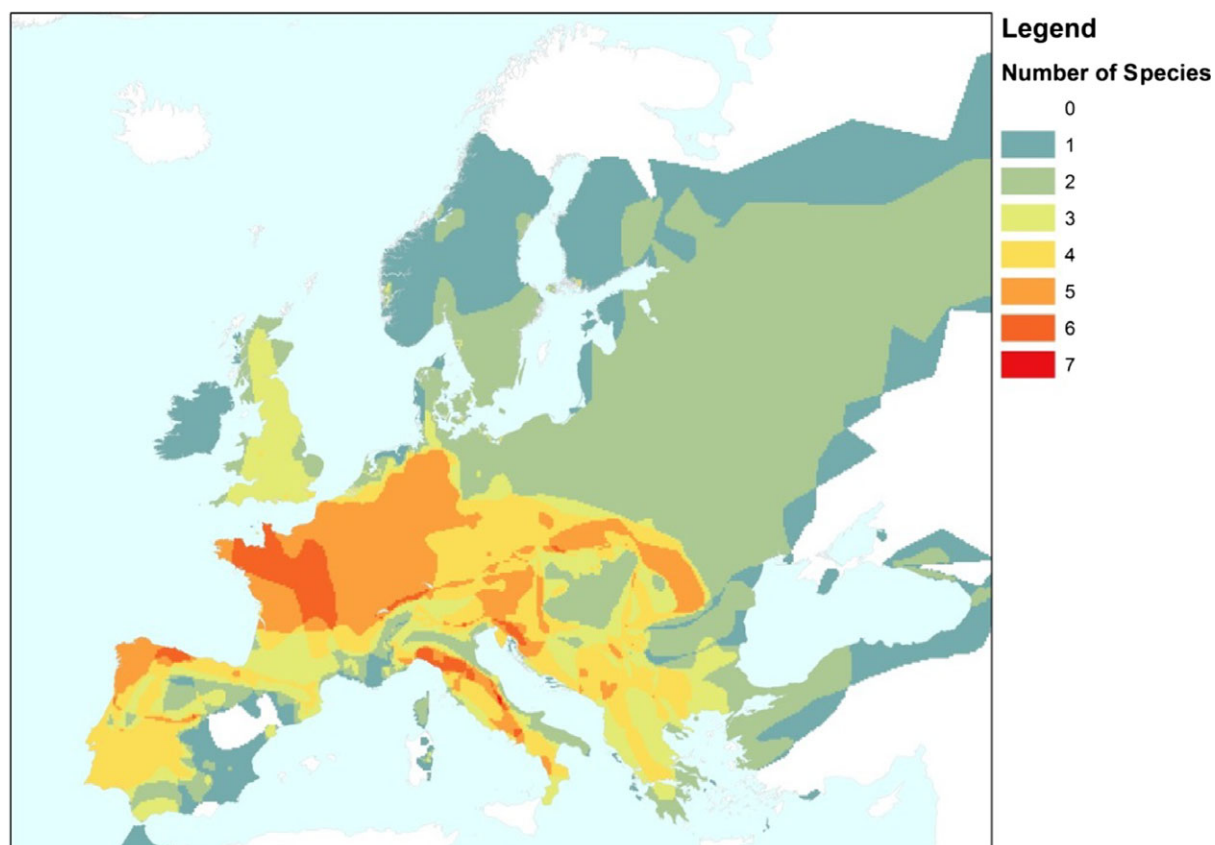


Figure 1: Map of the salamanders species distribution in the EU (data source: IUCN Red List; status: 11.11.2017) in which the number of species that occupy the same area/zone is represented by different colours. As shown in the figure, up to seven different species of salamanders may occupy the same area/zone

3.1.1.2. Information on the habitat of the Caudata distributed within the EU

The European salamander species known to be susceptible to infection by Bsal (Appendix E of EFSA, 2017) have variable habitat preferences and also differ in their dependency on the aquatic environment.

The IUCN website¹² was used for collecting the general information on salamander species described below; additional references are reported when relevant.

The salamanders are in general nocturnal during the terrestrial phase and diurnal or with continuous activity in the aquatic phase. Land salamanders can be active during daytime in rainy weather. Migration of adult salamanders from land habitats to water occurs in most species before reproduction over distances of several hundred metres (Kovar et al., 2009). The duration of the aquatic phase of adults varies depending on the species. Juveniles usually leave the water shortly after

¹² <https://www.iucn.org>

metamorphosis. Although several species are often found at the same site, their habitat use throughout the year may differ significantly.

Salamandra salamandra is a broadly distributed species usually present in wet cool forests with shaded brooks, small rivers or forest ponds, preferring humid microhabitats with dense leaf litter and moss. Mating occurs on land and females only visit aquatic habitats to give birth to larvae, that complete metamorphosis in water. Two subspecies are fully terrestrial giving birth to metamorphosed young. *Salamandra salamandra* overwinters in underground refuges that protect the animals from freezing, suitable sites can be used by multiple individuals repeatedly for several years. It can coexist with several other salamander species on the same site (e.g. *Ichthyosaura alpestris*, *Lissotriton* sp., *Triturus* sp.).

The genus *Speleomantes* is present in Europe with eight species, each with a very localised distribution. All species are fully terrestrial and live in humid rocky outcrops, caves, crevices and forested areas in the vicinity of streams, usually on sites with dense growths of moss. All species reproduce by a few directly developing terrestrial eggs, not visiting water bodies.

Triturus cristatus inhabits forests, pastures, meadows, parks and gardens. It reproduces in ponds or semi-flowing waters. Adults spend around 5 months in the aquatic habitat, but most individuals overwinter on land.

Lissotriton italicus is often associated with woodland and agricultural habitats. It breeds in temporary ponds, ditches, slow-moving streams and lakes. It is also found in modified habitats, such as wells and water tanks. Adults spend several months in aquatic environment, but aestivate (summer dormancy) and hibernate on land.

Lissotriton vulgaris is the species with the broadest distribution among European salamanders and it is an adaptable species occurring in woodland habitats, meadows, parks, gardens, various damp habitats as well as in rural and urban areas. The species breeds in still and slow-moving shallow waters and irrigation ditches. Mating and reproduction occur in water, adults remaining in the aquatic phase for several months. This species hibernates on land. Often a single hibernation site is used by a large number of adults and juveniles (Baruš and Oliva, 1992).

Ichthyosaura alpestris is a predominantly aquatic species generally found close to water, inhabiting forests, subalpine meadows and pastureland. The species is not very selective in choosing breeding sites, provided they are not stocked with predatory fish. Mating and larval development takes place in variety of stagnant waters and sometimes in slow-moving streams. Hibernation occurs on land, but larvae sometimes overwinter in water before metamorphosis in the following season (Baruš and Oliva, 1992). Fully aquatic neotenic (adults retain larval gills) populations occur in some areas (IUCN, online).

Pleurodeles waltl is a highly aquatic species of Mediterranean-type habitats including scrub, open woodland and cultivated land. It is generally found in water bodies with dense aquatic vegetation. It is adapted to survive on land using shelter when the aquatic habitat dries up. Hibernation takes place in the aquatic habitat.

Euproctus platycephalus inhabits mountainous areas with permanent and temporary stagnant and running waters; in rivers, it prefers calm areas. Its terrestrial habitats are generally restricted to riverine scrub or woodland, and the species may also be found in cave systems. Its breeding sites are permanent pools, water holes, small lakes and streams. The eggs are deposited between stones or are buried in sand; the larvae develop in the streams (Griffiths, 1996). The species is quite adaptable and can be found in artificial pools. Hibernation takes place in the aquatic habitat.

Salamandrina perspicillata is mainly found in forests with dense undergrowth in hilly and mountainous areas. Only females of this species are aquatic during the short oviposition phase for which well oxygenated waters, slow running streams or small ponds are used (IUCN, online).

3.1.1.3. Identification of EU zones that may be considered infected with Bsal

Wild salamanders

In the EU, a large proportion of genera of the family Salamandridae and the genera *Speleomantes* of the family Plethodontidae has been shown to be wildlife species naturally and/or experimentally susceptible to Bsal. In addition, Bsal has been shown to cause very high mortality in some species (e.g. *Salamandra salamandra*) (for reference, see EFSA AHAW Panel (2017) and EFSA (2017)). In the species *Salamandra salamandra*, introduction of Bsal leads to a fast population collapse (Martel et al., 2013; Spitzen-van der Sluijs et al., 2013; Stegen et al., 2017).

To date, Bsal obvious mortality has been detected only for certain salamander species (e.g. *Salamandra salamandra*; for more details, see EFSA (2017)). However, infection of wild anurans with

Bd has been associated with both mass mortality events but also less obvious, slow, and in some cases cryptic, population declines even at very low prevalence of infection, after the disease has become endemic (e.g. Murray et al., 2009; Pilliod et al., 2010; Valenzuela-Sanchez et al., 2017).

In Belgium, no Bsal was detected via active surveillance. All Bsal-positive sites (Eupen, Dinant, Liège and Robertville) were detected via passive surveillance (in *Salamandra salamandra*). Bsal infection loads were monitored for 2 years after the detection of a Bsal outbreak in Robertville (Stegen et al., 2017). No more *Salamandra salamandra* (juveniles or adults) have been found at the outbreak sites in Eupen in 2017 (outbreak detected in 2013) and Robertville in 2017 (outbreak detected in 2014). In the outbreak site in Bunderbos, in the Netherlands (first Bsal-positive animals detected in 2010 via retrospective testing after discovery of the pathogen), *Salamandra salamandra* tested negative for Bsal in 2015 (2 animals) and in 2017 (35 animals).

Screenings in the UK in wild populations were carried out in 2014–2017 and no Bsal was detected (unpublished data, Andrew A. Cunningham, Institute of Zoology, Zoological Society of London, personal communication, 2018). Also, the results of the surveillance in the Czech Republic were all negative (Baláz et al., 2018).

No screenings have been performed in Italy and Sweden.

The results of the surveillance activities carried out in some European countries (see also Section 2.2.2.2) are summarised in Figure 2a as a result of what is currently known about Bsal presence in Europe. Some of the data are still unpublished (see also Appendix B). No information on Bsal screenings or surveillance activities is available from the remaining MSs.

Kept salamanders

In addition, the disease has been detected in collections of captive salamanders in Belgium, Germany, Spain, the Netherlands and the UK (see Figure 2b; Sabino-Pinto et al., 2015; Fitzpatrick et al., 2016, In Preparation). The salamander species that have died due to confirmed and natural Bsal infection in captivity are: *Ichthyosaura alpestris*, *Salamandra algira*, *Salamandra salamandra* (including the subspecies *alfredschmidtii*, *almanzoris*, *bernardezi*, *fastuosa*, *gallaica*, *gigliolii*, *salamandra*, *terrestris*, *wernerii*), *Salamandra atra*, *Salamandra corsica*, *Salamandra infraimmaculata*, *Triturus macedonicus*, *Triturus marmoratus*, *Notophthalmus viridescens*, *Taricha granulosa* and *Lissotriton boscai* (Sabino-Pinto et al., 2015; An Martel and Frank Pasmans, University of Ghent, personal communication, 2018).

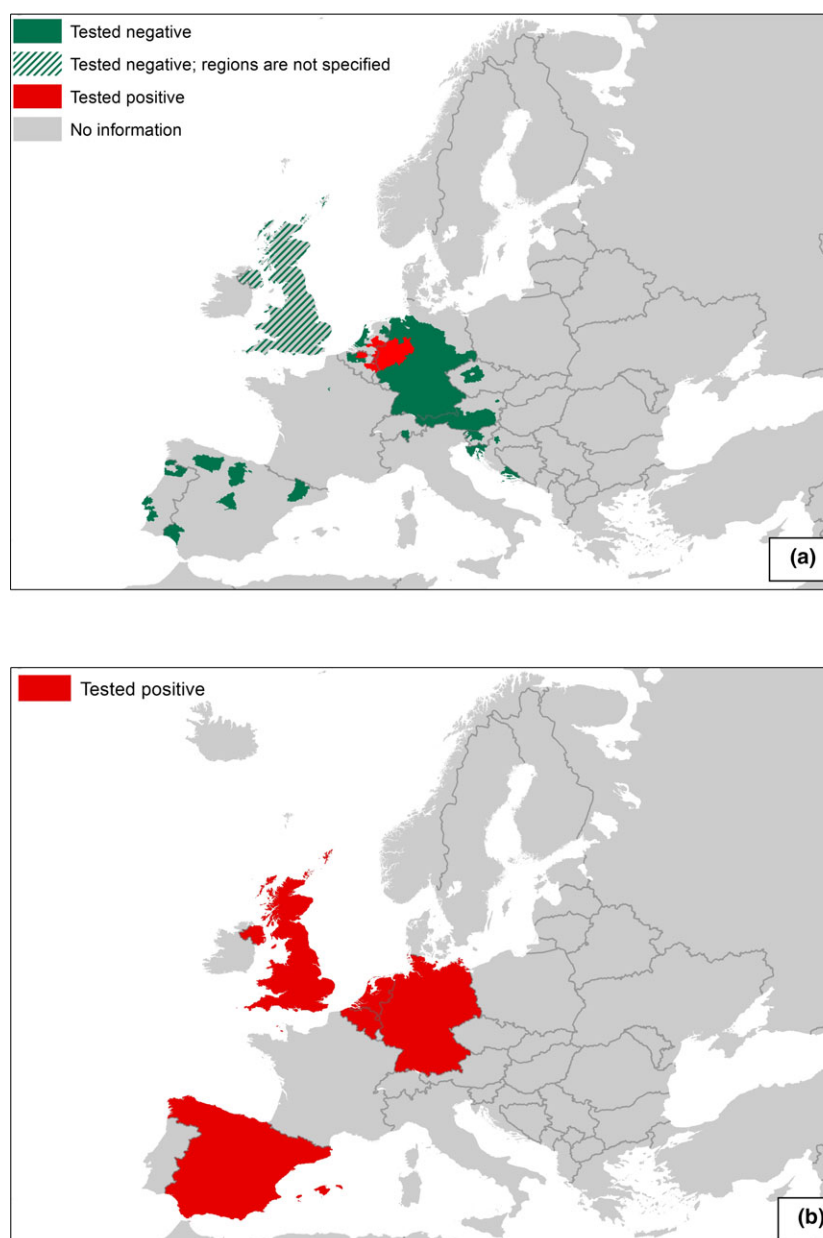


Figure 2: Maps with current knowledge of Bsal in Europe based on the screening and surveillance activities. (a): Actual distribution of where Bsal has been confirmed to be present (red colour) and where the surveillance systems have not confirmed its presence (green colour) in wild populations of salamanders: if in the given period of time at least one individual (without considering the species) has been Bsal-positive tested, the region/area has been considered as positive (red-coloured). In grey the areas/regions where no information is available. Data and results of the surveillance activities carried out in Austria, Belgium, Croatia, Czech Republic, France, Germany, Portugal, Spain, Slovenia, Sweden and the Netherlands are detailed in Appendix B. (b): EU MSs where collections of salamanders in captivity have been Bsal tested positive

The only existing detailed analysis of disease and population dynamics in an affected wild subpopulation was recorded in Robertville (BE) and is described by the disease monitoring in Figure 3 showing frequency of infected and uninfected salamander detections through time since the first recording of the outbreak.

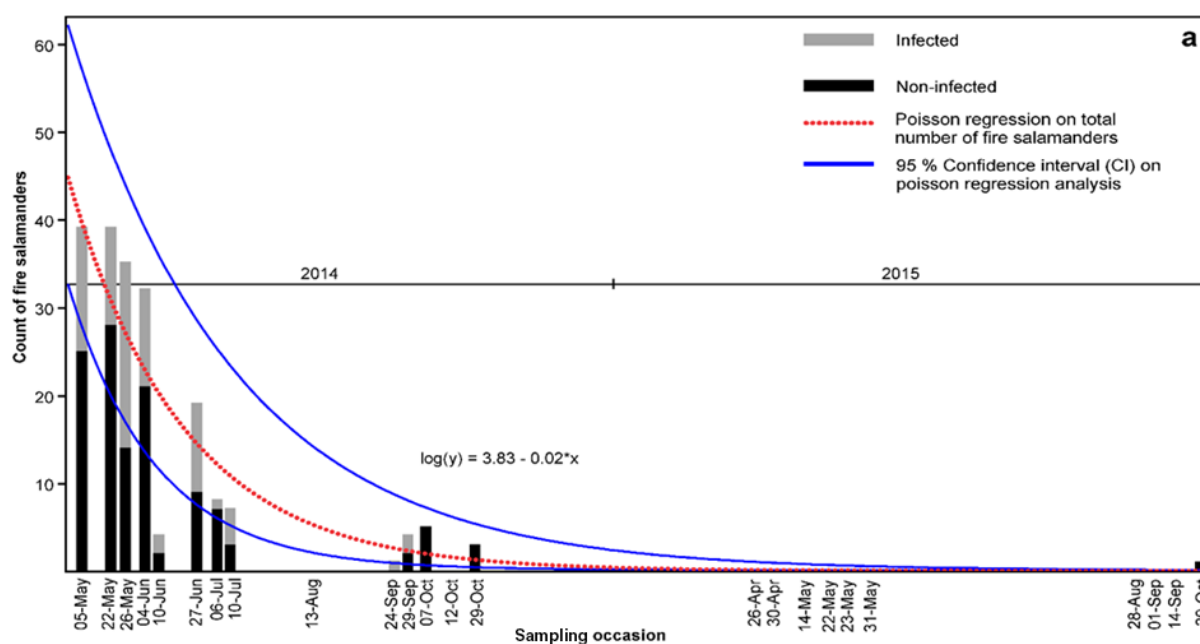


Figure 3: Frequency distribution of Bsal-infected and Bsal-uninfected individuals of *Salamandra salamandra* found per visit in Robertville (BE) beginning with the detection of the outbreak in 2014. Overtime the decline of the probability to detect even any salamander was modelled with the Poisson distribution. As can be observed, the size of the population dramatically decreased within a few months. The two clusters of positive findings relate to the main activity periods of the hosts. (Reprinted by permission from: Springer Nature, Nature, Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*, Stegen et al., 2017 ©)

Predictions generated from climatic niche modelling based on native Bsal records suggest the possibility of an extensive distribution across Europe, in addition to parts of the North African Mediterranean shore and Anatolia. The distribution range of every salamander species in Europe is likely to overlap with climate conditions that are suitable for Bsal. The current Bsal distribution appears to be only a small fraction of the potential distribution as predicted by niche modelling. This suggests that invasive Bsal could continue to spread and expand its distribution in Europe and pose an increasing risk to salamander diversity across the Western Palearctic (Beukema et al., submitted). Climate conditions suitable for Bsal modelled earlier in Feldmeier et al. (2016) also support the possibility of further Bsal spread in Europe.

However, the assumed distribution range of salamander species is based on a gross spatial scale. Therefore, small-scale salamander habitat preferences may actually limit the total overlap between Bsal and salamanders. The assessment could be refined using an ecological niche model of salamanders including small-scale habitat categories, such as vegetation characteristics, wetland distribution, ecological corridors (Guevara et al., 2018). However, these data are not currently available.

3.1.2. Suitability of surveillance methods to ensure reliable and robust demonstration of presence or absence of Bsal (ToR 2d)

3.1.2.1. Protocol for Bsal diagnosis

In addition to a case definition for infection with Bsal (White et al., 2016), several diagnostic methods for Bsal detection have been described, including histology/histopathology, immunohistochemistry, PCR, qPCR and lateral-flow technology (Bloo et al., 2013; Martel et al., 2013; Dillon et al., 2017; Thomas et al., 2018). PCR and qPCR are currently the only diagnostic methods that can distinguish between Bsal and its sister species Bd.

The Bsal qPCR developed by Bloo et al. (2013) has the advantage of simultaneously identifying and quantifying Bsal in amphibian samples. Conservative values of test sensitivity and specificity under field conditions were proposed previously (EFSA, 2017; EFSA AHAW Panel, 2017); Bsal-specific qPCR

assay detects 0.1 Bsal genomic equivalents (GE) and has shown an average experimental sensitivity ranging from 96.2% to 100% and an average specificity of 100% (Thomas et al., 2018).¹³ This qPCR can be performed on non-invasively collected skin swabs and the test is able to detect the fungus before the animal shows clinical signs of disease (Martel et al., 2014). Non-invasive sampling protocols that have been developed for Bd diagnosis (Hyatt et al., 2007; Skerratt et al., 2008) are currently used to collect samples from live wild and captive amphibians for testing the presence of Bsal.

The test sensitivity of qPCR should be used to estimate the sample size needed for any surveillance purposes.

The EC has launched a call for tender (ENV.B.3/SER/2016/0028) and it is setting up an EU network of diagnostic centres. Laboratories in Austria, Belgium, the Czech Republic, Croatia, France, Germany, Italy, Slovenia, Spain, Sweden and the UK have participated and successfully completed the Bsal ring test. The list of these laboratories is available online¹⁴ and it will be updated as new laboratories participate in the testing.

For any salamander species, diagnosis with Bsal-specific real-time PCR needs to be performed over the last week of any quarantine and at the moment of handling a free-living or kept individual if surveillance takes place.

3.1.2.2. Bsal surveillance

Surveillance of wildlife diseases in an area, zone or region (i.e. population unit) has different goals consequent to the acute knowledge about the disease in this population unit. With Bsal, the goal is to detect at an early state its introduction into or its spread within the unit. Subsequently, the goal is to monitor the salamander population known to be Bsal affected until eventually no more Bsal-infected animals are sampled, and thereby document absence of the pathogen in the population unit. This situation-oriented surveillance concept was shown to be purposeful for other wildlife disease surveillance planning, in particular for efficacy and resource requirements (Thulke et al., 2009). The following paragraphs consider the proposed surveillance activities (passive vs active) in the context of Bsal and identify beneficial protocols, drawbacks and purposeful interpretation following diagnostic results derived for a population unit. Conceptually, the epidemiological absence of the pathogen from a population unit is considered different from a 'freedom from disease' approach, ascertaining prevalence unlikely to be above a certain detection threshold.

Passive Bsal surveillance

Passive surveillance consists of opportunistic detection of dead individuals and testing of these for Bsal infection. It implies that all salamanders that are found dead need to be Bsal tested.

Entry of Bsal into a habitat area or subpopulation of European host species is associated with substantial mass mortality in the animals. On the basis of the surveillance activities currently in place in some MSs (see Section 2.2.2), and considering the lack of information on the geographical structure of salamanders' population prerequisite to a valid sampling scheme in live salamanders, passive surveillance was considered as the surveillance system most fit for the timely detection of Bsal emergence in wild host populations.

To enhance the procedure in context of Bsal independent visits of host populations, a protocol should be implemented that rises awareness of dead animals and gives details for the subsequent delivery of Bsal exclusion diagnosis, including the methodology for packing the samples i.e. dead animals.

If people find a dead animal, they should immediately alert the contact point/disease emergency team for collecting the samples. The contact point is in charge of correctly collecting the samples and bringing to a laboratory for the testing and georeferencing the findings.

Practical information for collecting the samples is reported for Bd (Murray et al., 2011a).

¹³ The study investigated the test sensitivity of different Bsal testing tools in four different laboratories. The first three laboratories used Bsal-specific real-time PCR (qPCR) for detecting Bsal, whereas the fourth used droplet digital PCR (ddPCR). For qPCR, the average test sensitivity was: laboratories 1 and 2 = 100% (confidence level (CL): 95% and confidence interval (CI): 86.6–100%); Laboratory 3 = 96.2% (CL: 92.6%; CI: 80.4–99.9%), whereas the laboratory 4 showed a lower average test sensitivity = 92.3% with CL of 95% and CI of 75–99%. This difference was considered to be due to the different technique that was used in the fourth laboratory.

¹⁴ https://docs.wixstatic.com/ugd/1ecffe_b77e2c99423844c191f61e6ecb478fb9.pdf

Active Bsal surveillance

Active surveillance can be defined as a systematic check of all local populations (subpopulations) in the wild for sentinel purposes and testing for Bsal. Skin swab samples have to be collected from randomly selected live wild salamanders. Detection of Bsal emergence would be as early as the temporal schedule of the visits prescribes. To date, mainly non-systematic active surveillance activities exist covering the host population of Bsal in the EU MSs; the main limitations for a Bsal surveillance strategy regard the lack of data on host population, size and distribution. However, possible reasons might be related also to the fact that the activity would require substantial resources to become sufficiently sensitive, issues with physical accessibility of subpopulations and implications for unintentional translocation of the fungus due to necessary additional visits. In summary, a systematic active surveillance strategy cannot be considered the first choice for Bsal detection due to feasibility and practicality issues.

If a Bsal outbreak has already been detected, however, active surveillance and monitoring of Bsal dynamics may be advised for, e.g. appropriate conservation decisions.

If active surveillance is planned, overarching protocols are suitable (Wobeser, 2007) and the following steps apply to Bsal:

- Sample definition
- Where to sample?
- When to sample (seasonality)?
- How many samples should be taken (sampling size)?
- Which species should be sampled?
- Which diagnostic test should be used?

Sample definition

The sample should consist of skin swabbing from live wild salamander populations. Only people with the specific permit can carry out this activity.

Where to sample?

Terrestrial salamander species (e.g. *Salamandra salamandra*), and newt species during their terrestrial life stage, can be sampled, either by active search or by using pitfalls. For aquatic life stages, dipnets or aquatic traps (fikes) can be used to catch newts in suitable reproduction habitats (depending on species: ponds, streams, etc.).

When to sample (seasonality)?

The detection of Bsal is much related to the retrieval of the hosts; therefore, for practical reasons, the surveillance activities should take the natural activity of salamanders into account. For terrestrial Caudata, disease surveillance is useful only during activity periods, which are highly dependent on local climatic conditions and the Caudata species involved (e.g. for many terrestrial salamanders in Mediterranean systems: activity mainly dictated by humidity, in temperate and high elevation regions by humidity and temperature). For aquatic Caudata, sampling should be confined to the aquatic activity season, which varies according to species, habitat and local climatic conditions (e.g. in high elevation species: activity peak in the summer, Mediterranean climate: often in the winter; temperate climate: often in the spring).

How many samples should be taken (sampling size)?

In Table 3 of EFSA AHAW Panel (2017), the calculations of the sample size¹⁵ were provided for the conservative assumptions of 80% test sensitivity under field conditions based on the laboratory estimates of diagnostic sensitivity of about 96% (Thomas et al., 2018).

The calculations should consider all the susceptible, tolerant and reservoir species present when the target population size is determined. Considering that reservoir species are less reliable for Bsal detection, the sampling should put preferences on susceptible species. In any case all salamanders that are found dead should be tested.

¹⁵ For providing the 95% probability of detecting at least one positive animal according to different population sizes and minimum expected prevalence of 1%, 3% and 10%.

Which species should be sampled?

On the basis of the current available knowledge, individuals of all the susceptible, tolerant and reservoir species should be included. However, in tolerant and reservoir species, infection may have low intensity (DiRenzo et al., 2017). If there is a particular susceptible species present, sampling effort should be focused on this species.

An overview of the salamander species that are considered Bsal susceptible, tolerant and reservoir is provided by EFSA AHAW Panel (2017). The same document reports a list of salamander species that are suggested to be listed for Bsal according to the criteria of Art. 8(3) of the Animal Health Law.¹⁶ Among these species, those reported present in the wild in the EU should be included in an EU surveillance system.

However, the experts highlighted that the species susceptibility cannot be the only element on which to base a surveillance system. The other fundamental items to be taken into consideration for recommending which salamander species would be the most relevant for inclusion in surveillance systems are: inclusion in the Annexes of the Habitats Directive, potential points of entry, range and population sizes, and species distribution (Nusser et al., 2008).

Small-range species with small population sizes are predicted to be the most vulnerable to extinction processes (Sodhi et al., 2008) and together with species already classified as threatened (e.g. by IUCN or at national level) should be considered of high priority. Widespread and abundant species can be used as sentinel species (e.g. *Salamandra salamandra*).

Recommendations should be made on a risk analysis per species. However, currently, there is not enough knowledge on the items mentioned above and a risk analysis leading to a prioritised list of European Caudata species is pending. For this reason, the European Commission has launched a call for tender 'Mitigating a new infectious disease in salamanders to counteract the loss of European biodiversity' to get information/data on possible emergency action plans, including species prioritisation. The project involves seven partners, it started in March 2017 and is planned to be finalised by March 2020.

Which diagnostic test should be used?

Bsal-specific qPCR (see Section 3.1.2.1).

Demonstration of Bsal absence

Establishing the absence of disease can, in theory, help focus risk-mitigation actions by restricting them to those that may prevent invasion/establishment in areas with the known or suspected absence of the disease. For example, such demonstrations were a key reason underpinning the decision to impose a complete trade ban on salamanders in the USA and some authors have used absence of detection to suggest appropriate management steps at a regional scale as well (e.g. Wang et al., 2017).

To put in place a surveillance system to demonstrate the absence of a pathogen, the host population should be known and access to all host populations needs to be regularly advised. For Bsal, the knowledge of the salamander population is extremely poor. Available data are insufficient to monitor the presence of Bsal or demonstrate its absence. Moreover, assumptions on population size or on background prevalence, both necessary as an input to statistical or sample-based absence demonstration, cannot be formulated with purposeful certainty.

Sampling activities to demonstrate the absence from Bsal in the wild suffer practicality issues. Finding enough animals within a given habitat (e.g. 1 km²) to satisfy sample size requirements, very likely turns impractical; e.g. the sample size needed for providing the 95% probability of detecting a design prevalence of 1% would be 373 animals, assuming an infinite population size, a diagnostic test sensitivity of 80% and diagnostic test specificity of 100% (see Table 3 in EFSA AHAW Panel, 2017). Moreover, the target population unit that justifies the proposition of a proper design Bsal prevalence value is not yet defined. Even if no positive samples are found in a specific area/zone, it cannot be stated that Bsal is absent in that specific area/zone; it just means that no positives have been sampled in the tested population. In conclusion, the epidemiological demonstration of Bsal absence in wild populations of salamanders may be considered unfeasible.

A more purposeful approach assumes the absence as long as no Bsal-infected population has been detected by enhanced passive surveillance activities. Bsal infection is leading to substantial mass morbidity and mortality in infected populations and therefore falls under such surveillance concepts

¹⁶ Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law'). OJ L 84, 31.3.2016, pp. 1–208.

(Thulke et al., 2009). For such pathogens/diseases, enhanced surveillance focusing on individuals found dead is the most effective indication of their presence. Passive surveillance is suggested for early detection; therefore, if enhanced passive surveillance remains negative, the assumption of Bsal still being absent is justified.

Alternatives in kept salamanders

In a 'closed population' of susceptible salamander species kept in captivity, the owners have access to each individual. Therefore, a whole population testing would in principle be feasible. In that sense, it is considered feasible to demonstrate absence of Bsal by sufficiently long quarantine and absence of clinical symptoms of Bsal confirmed by visual observation. This should be complemented by testing all animals at the end of a quarantine period (e.g. 6 weeks for *Salamandra salamandra*, see Section 3.2.3.3). In this scenario, such epidemiological freedom from Bsal can be maintained when exchanges are limited to confirm Bsal-negative animals from other 'closed populations'. Premovement health certification should accompany the translocated animals (see Section 3.2.3.3) and the absence of clinical symptoms of Bsal should be confirmed by visual observation. The above-mentioned scenario is conditional on appropriate implementation of hygiene procedures and good practices (see Section 3.3.2).

In the scenario of 'close populations', the use of a sentinel, a susceptible individual to be cohoused during the quarantine, has been considered as an alternative to Bsal testing of all the salamanders at the end of the quarantine. Possible sentinel species are *Salamandra salamandra* and other hyper susceptible species (see Martel et al., 2014). However, this possibility should be further explored as currently there are not enough data to support the use of sentinel animals in the detection of Bsal presence/demonstration of Bsal absence. In addition, the possible inter-species toxicity should be carefully considered before co-housing different salamander species.

3.2. Spread of Bsal in and from infected areas or via infected animals or fomites (ToR 3)

3.2.1. Protocols for Bsal disinfection and treatment

3.2.1.1. Bsal disinfection

An overview of effective disinfection protocols is provided in Van Rooij et al. (2017).

The procedure for disinfecting equipment, fomites and other items that might have been contaminated by Bsal, after their cleaning, consists of: 1% Virkon S[®], 4% sodium hypochlorite and 70% ethanol for disinfecting equipment in the field, lab or captive setting, with a minimal contact time of 5 min for 1% Virkon S[®] and 1 min for the latter disinfectants (Van Rooij et al., 2017). To treat Bsal-contaminated materials, the use of high temperatures has also been proposed (EFSA AHAW Panel, 2017).

3.2.1.2. Treatments of Bsal-infected animals

Depending on the species, the infected salamander can be treated by an elevated environmental temperature (heat treatment) or with antimicrobials. Post-treatment assessment of Bsal absence is necessary and the treatment may need repeating until total clearance of Bsal infection.

Heat treatment

Ten days exposure to 25°C clears salamanders from Bsal infections (Blooi et al., 2015a). The main disadvantage linked to temperature treatment is that elevated temperature might not be endured by all salamander species, and may result in thermal shock. The occurrence of Bsal in (sub)tropical regions such as Okinawa (Japan) and northern Vietnam at temperatures above 25°C suggests the existence of Bsal strains with higher thermal tolerance (Laking et al., 2017). It might therefore be possible, that some Bsal strains could survive heat treatment at 25°C, although this has not been reported so far. Currently, there is no evidence of the presence of heat-resistant strains in Europe.

Heat treatment can be applied to the thermo-tolerant species of salamanders (e.g. *Triturus dobrogicus*, *Ommatotriton vittatus*, *Salamandra immaculata*, *Cynops orientalis*, *Paramesotriton hongkongensis* and *Salamandra salamandra*; see details and references in Blooi et al., 2015a; EFSA AHAW Panel, 2017; An Martel and Frank Pasmans, University of Ghent, personal communication, 2018), also as a preventative measure.

Treatment with antimicrobials

For the species of salamanders that are not thermotolerant, an alternative treatment is combining the topical spray application twice/day of voriconazole (12.5 µg/mL) with polymyxin E (colistin) submersion bath (2,000 IU/mL) and keeping the infected salamanders at 20°C for 10 days (Blooï et al., 2015b), whereas the antimicrobial treatment at an ambient treatment of 15°C failed to clear Bsal (Blooï et al., 2015b) (for other details see also EFSA AHAW Panel, 2017). In one rare example of field treatment of anurans, sporadic application of antifungal agents was not sufficient for the long-term and large-scale control of Bd (Geiger et al., 2017). Unintended side-effects also need to be evaluated; for example, Rohr et al. (2017) reported that antifungal treatments considered effective for killing Bd in culture may not be effective on wild amphibians and in some cases could exacerbate Bd-induced mortality.

Colistin is a polymyxin antibiotic that is considered as the final line of treatment for multidrug-resistant Gram-negative bacteria (Li et al., 2006) and exposure to colistin is associated with an increased risk of isolating colistin-resistant Enterobacteriaceae (Drozdinsky et al., 2017). Also the use of azoles-based antifungals (the most frequently used classes of antifungal therapy) may induce resistance in fungi (Fuentefria et al., 2017). Therefore, the preventative use of these antimicrobials in salamanders, which have not been confirmed to be Bsal infected, cannot be justified.

For those species that are thermotolerant, heat treatment is the preferred option; however, information about thermotolerance for individual species is incomplete.

3.2.2. Risk of survival, spread and establishment of Bsal within already infected areas and spread from infected areas into other parts of the EU by natural movements of wild live salamanders taking into account especially relevant geographical, hydrographical and meteorological conditions (ToR 3a–i)

From a biological point of view, the geographical unit can be defined as a patch of suitable habitats inhabited by the species in question.

For salamanders, defining the relevant geographical unit is difficult because many aspects are not known, and there is great variability among the species of salamanders. Based on the few data available and the experts' knowledge, it is suggested that, in most species, sites of occurrence separated by unsuitable habitat at a distance above 1 km can be considered as separate local populations with respect to pathogen spread.

At this moment, it is not known if the outbreaks that occurred in the EU in wild salamander populations were somehow linked; there is a lack of knowledge about the possible sources of pathogen contamination within the different Bsal-positive sites. Some studies are currently carried out in the south of Belgium. Areas where Bsal has been detected are relatively close, in a specific region (although in three countries: BE, DE, NL) and are sites frequented by people including those interested in salamanders. This might suggest a possible route of introduction from one site to another (or several introduction events, to be shown by genetics studies).

Direct Bsal transmission can occur between individuals of the same species of salamanders (intraspecies: Martel et al., 2013), of different salamanders' species (interspecies: Martel et al., 2014) or between anurans and salamander species (Stegen et al., 2017).

The capability of Bsal to be transmitted directly via infected animal contacts, or indirectly via healthy carrier animals by encysted spores, motile zoospores or contaminated soil has been already reported (EFSA AHAW Panel, 2017).

Several carriers could have a role in transmitting Bsal to salamanders: e.g. Bsal adherence of spores on goose feet have been shown experimentally (Stegen et al., 2017). Also, frogs and toads could be Bsal carriers (Nguyen et al., 2017; Stegen et al., 2017) and these amphibians can move from hundreds of metres up to several kilometres during migrations (Tunner, 1991; Kovar et al., 2009). In addition, waterfowl carrying spores of the sister species Bd on skin or feathers have been reported (Wimsatt et al., 2014).

Bsal produces two types of spores (see Figure 4). The motile 'spores' actively swim in water searching for host skin, and they are predated upon by zooplankton. The 'encysted non-motile spores' are resilient and thick-walled; they float, remain infective for at least a month in water and are more resistant to predation by zooplankton. Bsal present in wet soil remained infective up to 48 h after the soil was contaminated by an infected salamander (Stegen et al., 2017). The fungus can survive on subclinically infected salamanders and frogs for several months and these can act as active or passive carriers (Martel et al., 2014; Stegen et al., 2017).

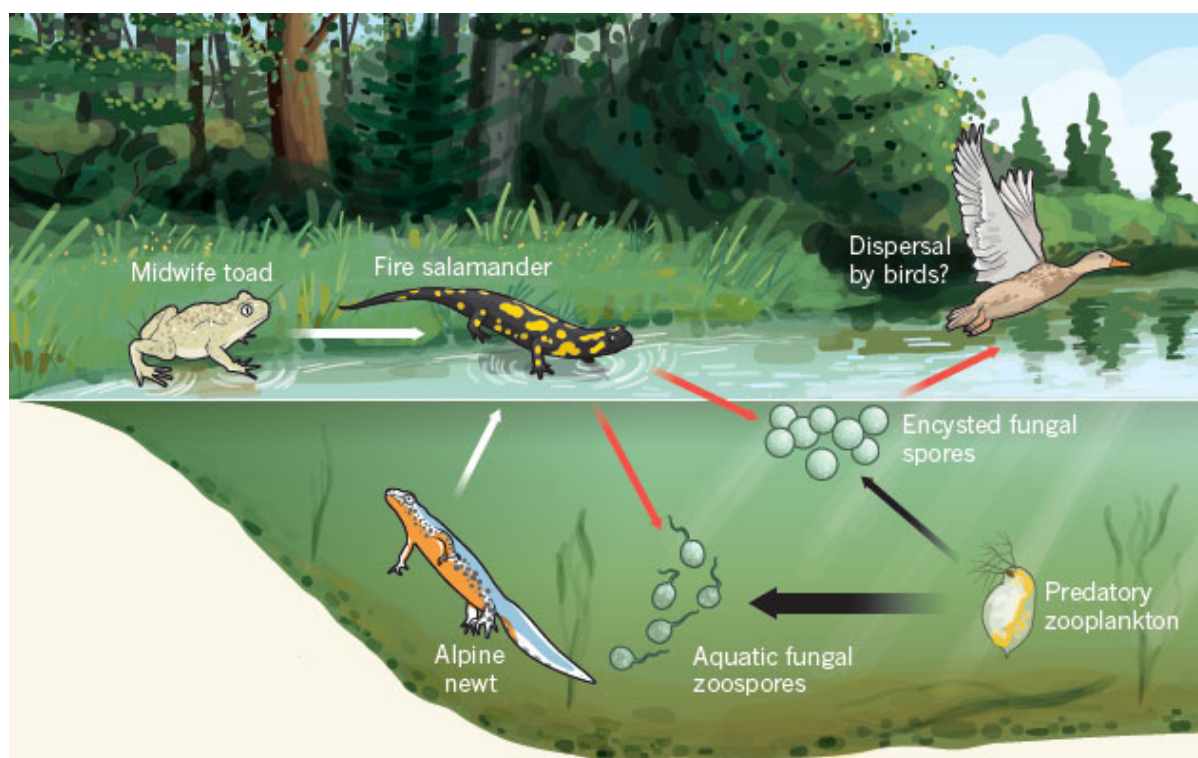


Figure 4: Description of the possible means for Bsal environmental persistence and spread in the wild (see also description of the means of spread in the text below) (Reprinted by permission from: Springer Nature, Nature, In peril from a perfect pathogen, Fisher, ©2017)

Among all the possible means of Bsal spread, the ones that, depending on the scenario, were considered as the 'top three means' by the experts are listed below, with a short explanation of the rationale behind the result of the qualitative categorisation. As explained in Section 3.2.4, the contributors to Bsal spread can act as carriers of the fungus.

Scenario A – Main contributors to Bsal spread and survival in an infected area within a subpopulation:

- Salamanders intraspecies: individuals of the same species have a high role in actively transmitting Bsal because they have high probability to meet and interact (mating, hibernation), even more so than between different salamander species.
- Salamanders interspecies: direct Bsal transmission between individuals of different species can also be relevant. However, there is variability in the susceptibility to Bsal among different species.
- Frogs and toads (Anura): Bsal has been experimentally shown to be transmitted directly from an anuran species to salamanders, and Bsal has been found to be naturally present in one anuran species. However, the contribution of this means of transmission of Bsal to salamanders has been ranked less probable by the experts because it has not been fully studied: e.g. it is not known if other anuran species may be active or passive carriers of Bsal. In addition, the probability that salamanders come in contact or interact with anurans is lower compared with intra-species or inter-species interactions among salamanders.

Scenario B – Main contributors for the spread of Bsal from an infected area to an uninfected area, between subpopulations of the same meta-population:

- Amphibian-related human activities: people (and related equipment) that during specific amphibian-related activities, e.g. for research purposes, may enter in contact with infected salamanders or their habitat and may move from one site to another even in a short time and over long distances. Transport of salamanders for conservation purposes, assisted dispersal and reintroductions can also be important processes in Bsal spread.

- Wild birds: movement of wild birds carrying spores can transmit Bsal from an infected area to uninfected areas because of the long distance that they can reach in a short time and due to the experimentally confirmed ability of spores to stick to bird's feet.
- Salamanders interspecies: infected individuals of a species with higher ability to cross unsuitable habitat or species transported (e.g. by flood, human activities) to new sites, can support spread from an infected site to a new one.

Scenario C – Main contributors for the entry of Bsal from an infected area to an uninfected area across geography barriers between meta-populations:

- Wild birds: movement of wild birds carrying spores can transmit Bsal between meta-populations (also across geographical barriers) because of the long distance they can fly in a short time.
- Amphibian-related human activities: people (and related equipment) that for specific amphibian-related activities, e.g. for research studies, may enter into contact with infected salamanders or their habitat, and may move from one site to another even in a short time and for long distances.
- Non-amphibian-related human activities: Bsal spores may attach to equipment used for other activities (e.g. fishing, forestry) in Bsal-infected environments (e.g. water bodies). However, these human movements are less linked to the salamander-inhabited microhabitat compared with the amphibian-related human activities.

The following means have also been identified by the experts as possible carriers (mainly passive ones) of Bsal (see Table 2) and have been scored high (even without being a part of the 'top three') in one of more scenarios, for the following reasons:

- Water: for aquatic and semi-aquatic salamander species, water may be an important means of transmitting Bsal zoospores from an infected individual to an uninfected one. In addition, zoospores may survive up to 31 days in the water. In scenario B, water was scored as probable if water streams are connected (see Table 2). If no connection exists, this means was considered improbable to have a role in Bsal transmission.
- Substrate: Bsal has been detected in naturally contaminated soil, and the role of infected soil in transmitting Bsal to uninfected salamanders has been demonstrated. In addition, Bsal-contaminated soil remained infective up to two days after exposure to infected salamanders.
- Wild mammals: Bsal spores may attach to mammals entering infected waterbodies and environments. In addition, some wild mammals (e.g. raccoons, otters, minks) prey on amphibians and, therefore, may have direct contact with infected salamanders. Wild mammals can move for relatively long distances (within geographical barriers) and may contaminate uninfected areas (e.g. water bodies). However, currently there is no evidence of spores' transmission via wild mammals.

Reptiles, fish and invertebrates have been also identified as possible means of Bsal spread (see Table 2); however, they have been considered less important in the Bsal transmission for the following reasons: evidence of spores' transmission is available for Bd only and these means of spread cannot reach long distances between salamanders' subpopulations or meta-populations. In addition, fish are strictly linked to the aquatic environment and cannot be in contact with terrestrial salamander species (e.g. *Speleomantes* sp., *Salamandra atra*). Reptiles are present in both aquatic and terrestrial environments; however the dehydration and increase in temperature that they experience when they heat up under the sun, are not favourable to Bsal survival.

In conclusion, according to the experts' judgement on risk factor ranking, the risk of Bsal spread in an infected population is maintained by intra-species contact of salamanders or potentially salamander interspecies and interamphibian interactions. Entry into new areas and populations is most likely to be caused by human activities (mainly amphibian-related) and potentially by wild birds (and by wild mammals and connected streams of water that carry Bsal between subpopulations of the same meta-populations), all of which are characterised by the possibility of long-distance movements and transmission.

In the following Table 2, the means of Bsal spread identified by the experts have been reported together with a short description/explanation and reference to the available evidence for a possible Bsal transport (if any). When no evidence supporting a possible Bsal transmission was available, reference to available studies on Bd have been added, because they have been shown to be close enough to allow some generalisations for basic biological aspects for both species of the genus *Batrachochytrium* (for more details, see EFSA, 2017). The experts' qualitative assessment of each means has also been included in the table.

Table 2: Possible means of Bsal spread in the salamanders' natural habitat and the experts' categorisation(*)

Possible means of Bsal spread	Description/explanation	Available evidence for Bsal or, if not available, for Bd	Ranking category assigned to the particular means considering its potential to spread the pathogen within a subpopulation	Ranking category assigned to the particular means considering its potential to spread the pathogen between subpopulations of the same meta-population	Ranking category assigned to the particular means considering its potential to spread the pathogen between meta-populations
Salamanders intraspecies (active carriers)	Direct random contact or contamination of the shared environment Mating, fighting, overwintering, migration routes	Experimental evidence in co-housed salamanders (Martel et al., 2013)	A	B (compared with anurans: salamanders have higher infection loads, but they move less)	C
Salamanders inter-species (active carriers)	Direct random contact or contamination of the shared environment. Predation, overwintering, migration routes	Experimental evidence In co-housed salamanders (Martel et al., 2014; Stegen et al., 2017)	Aa	Ab	C
Frogs and toads (active/passive carriers)	Direct random contact or contamination of the shared environment Predatory interactions (salamanders feeding on frog larvae), overwintering sites, migration	Experimental evidence of <i>Alytes obstetricans</i> transmitting Bsal to salamanders (Stegen et al., 2017) Natural presence of Bsal in <i>Bombina microdeladigitata</i> (Nguyen et al., 2017)	Ab	B (compared with salamanders: anurans have a lower infection loads, but they can move more)	C
Wild birds (passive carriers)	Attachment of spores on waterfowl and possible contamination of water bodies	For Bsal: Experimental adherence on goose feet (Stegen et al., 2017) For Bd: Feathers and feet of waterfowl in experimental and natural conditions (Johnson and Speare, 2005; Garmyn et al., 2012; Wimsatt et al., 2014; Hanlon et al., 2017)	B	A	A

Possible means of Bsal spread	Description/explanation	Available evidence for Bsal or, if not available, for Bd	Ranking category assigned to the particular means considering its potential to spread the pathogen within a subpopulation	Ranking category assigned to the particular means considering its potential to spread the pathogen between subpopulations of the same meta-population	Ranking category assigned to the particular means considering its potential to spread the pathogen between meta-populations
Wild mammals (e.g. wild boars, racoon-dog, mink, otter) (passive carriers)	Attachment of spores on mammals visiting infected waterbodies, amphibian predators. Contamination of water bodies	From experts opinion	B	Ab	Bb
Water (passive carriers)	Motile zoospores and non-motile spores in contaminated water	Bsal: Experimental infections with water containing zoospores, survival of zoospores (Martel et al., 2013, 2014; Stegen et al., 2017)	Ac	Ac if streams are connected; C if there is no connection	C (except for downstream: B)
Substrate (passive carriers)	Tracks of moving infected individuals, sites of mortality, contaminated soil as source of infection	Experimental infection with infected soil (Stegen et al., 2017) Detection of Bsal naturally contaminated soil (Stegen et al., 2017)	Ac	Not applicable (NA; because it is related to human movements)	NA (because it is related to human movements)
Amphibian-related human activities (passive carriers)	Movement of all above-mentioned means of transport (release of amphibians, movement of amphibians in conservation/research, aquaculture, ornamental ponds, etc.) due to human movements and equipment (e.g. boots) related to activities specifically linked to amphibians or their habitats (e.g. scientists)	For Bsal: Zhu et al. (2014) For Bd: The presence in farmed and human introduced invasive species (e.g. Fisher and Garner, 2007; Fisher et al., 2009; Reeder et al., 2012), in reintroduction programmes (Walker et al., 2008) In sold amphibians as fishing bait in USA (Picco and Collins, 2008); however, this practice is not common practice in EU	B	A	A

Possible means of Bsal spread	Description/explanation	Available evidence for Bsal or, if not available, for Bd	Ranking category assigned to the particular means considering its potential to spread the pathogen within a subpopulation	Ranking category assigned to the particular means considering its potential to spread the pathogen between subpopulations of the same meta-population	Ranking category assigned to the particular means considering its potential to spread the pathogen between meta-populations
Not amphibian-related human activities (passive carriers)	Human movements and equipment not specifically related to amphibians (forestry, agriculture, hunting, fishing, sports, etc.) that by chance get in contact with a contaminated source and transport Bsal spores. These activities can cause changes in amphibian population, movement, water flow, etc.	From experts opinion	Ba	B	B
Reptiles	Random attachment and transmission of spores on surface of reptiles (e.g. lizards, snakes, turtles)	Not for Bsal For Bd: attachment on reptilian skin (Kilburn et al., 2011)	B	C	C
Fish	Infection of fish	Not for Bsal For Bd: Experimental infection of zebrafish larvae, but no detection in wild fish (Liew et al., 2017)	B	C	C
Invertebrates	Infection or passive carriers,	Not for Bsal For Bd: Crayfish species in USA (McMahon et al., 2013)	B	B	C

(*): All means were ranked by their relevance for spread of Bsal in wild live salamanders and put in three different categories dependent on the following scenarios of spread: (i) within a subpopulation, (ii) from an infected area to an uninfected area between subpopulations of the same meta-population, and (iii) from an infected area to an uninfected area across geographical barriers (between meta-populations), by using a consensus discussion with categories from 'A' to 'C' which were coloured differently; 'A' (coloured in light blue) represents means that were categorised as most relevant for Bsal spread in a certain scenario, whereas 'B' and 'C' (coloured in yellow and red, respectively) were categorised of lower importance. If necessary, subcategories were introduced to express within category differences using small letters: a, b and c.

3.2.3. Risk-mitigating measures that could potentially be effective in ensuring safer international or intra-EU trade of live salamanders (both captured in the wild and bred) and their products and by-products as regards the transmission of Bsal, including diagnosis and potential treatment(s) (ToR 3b)

3.2.3.1. Possible salamander products and by-products

Salamander products and by-products are not known to be produced in the EU. Although in Asia, and particularly in China, there is a considerable use of newts and salamanders in traditional oriental medicine and for human consumption, no data are available to support the possibility that live Caudata or their (by-)products are being imported into the EU for these specific purposes (Tracey King, Ornamental Aquatic Trade Association Ltd, personal communication, 2018); however, the possibility that they might be imported for satisfying a niche of specific (mainly Asian) customers cannot be excluded.

In general, salamander species are of marginal importance for food consumption or other purposes, with single species as an exception: China has developed an intense giant salamander (*Andrias davidianus*) farming industry in recent years, with over 2.6 million animals held in official farms in 2011. The farms have limited if any biosecurity measures against introduction and spread of infectious disease. There are economic pressures on the provincial and national governments to remove the protected status of the species and to simplify licensing for the sale of Chinese giant salamanders and their products (Cunningham et al., 2016). The farms attempt to sell their products internationally, also online.¹⁷ *Andrias davidianus* is a CITES (Convention on International Trade in Endangered Species)¹⁸ listed species, therefore, the movements of this species are regulated. At present time there is no knowledge about importation of *Andrias davidianus* meat into EU. Data on Bsal infections in the species are missing, therefore the potential of Bsal spread by the salamander farming industry and its products is unclear.

In addition, even if illegal movements may exist, the experts consider it unlikely that the meat, which is produced for human consumption, would enter in contact with live salamanders or any aquatic environment within the EU. Therefore, the possibility that this product poses a threat to EU populations of salamanders is considered negligible.

Traditional oriental medicine

The skin, maw and bladder of salamanders (e.g. of *Andrias davidianus*; Still, 2003) are used in numerous remedies in China for the traditional oriental medicine.¹⁹ These animal by-products, however, derive from salamanders that have been heat treated at 25°C and desiccated. Therefore, they are not considered relevant for the potential spread of Bsal to the salamander populations in the EU.

Other by-products of amphibians such as leather or poison do not pose a threat of Bsal transmission as they are heat treated or dried (e.g. leather) or are not usually imported into the EU (e.g. poison).

Salamanders used as fish-baits:

Information exist on the trade of live salamanders used as fishing baits in some areas of the north of the USA (Picco and Collins, 2008), however there is no evidence of this practice being used in Europe and in some EU MSs it is likely prevented by national laws on species conservation and against cruelty to animals (CZ, DE, IR).

Considering the above reported information, it is concluded that heat treatment or desiccation of salamander products and by-products is feasible and effective to ensure safe international and intra-EU trade. Therefore, it was not considered relevant to include salamander products and by-products in the assessment reported in Table 3.

¹⁷ For example: <http://www.e-cantonfair.com/products/giant-salamander-meat-792802.html>: Chinese company that is selling Chinese products online.

¹⁸ <https://www.cites.org/eng>

¹⁹ <http://www.sciencedirect.com/science/article/pii/S0965229903000554>

3.2.3.2. Description of how live salamanders are transported in international or intra-EU movements

Most traded salamanders are shipped as adults or metamorphosed juveniles. Even if it is possible, it is unlikely that larval stage of Caudata, other than axolotls (*Ambystoma mexicanum*), is traded commercially within the EU. Other than axolotls, the only larval species that it is known to have been commercially traded is neotenic tiger salamanders (*Ambystoma tigrinum*) originating from the USA. However, there is no evidence that such commercial trade in neotenic tiger salamanders has taken place within the last 10 years (Tracey King, Ornamental Aquatic Trade Association Ltd, personal communication, 2018).

For international import to the EU, the transport of salamanders normally takes place by air, and, according to the current legislation (Council Regulation (EC) 1/2005²⁰) the transport by air should comply with the International Air Transport Association (IATA) Live Animal Regulations (LAR)²¹ standards for transportation of live animals.

For intra-EU movements, the transport may occur on road-vehicles under conditions that may vary according to several factors, e.g. distances, species, amount of animals (Paul Bakuwel, Ornamental Fish International, personal communication, 2018). When transported, salamanders are included in primary containers, which are different for aquatic or terrestrial species. The small perforated plastic boxes for the terrestrial species are filled with wet substrate (sphagnum moss, filter fibres, paper towels, etc.). The substrate maintains high humidity and protects the animal from injury. The container is placed in a cardboard box and an insulation box, to protect the animal from mechanical and thermal stress. If there is high ambient temperatures during transport, cooling with ice or cool gel packs is recommended. Primary containers of aquatic species are double plastic bags filled with water and a mixture of oxygen and air. Eggs or larvae of some aquatic species (e.g. *Ambystoma mexicanum*) are shipped in containers (or breather bags allowing oxygen diffusion) completely filled with clean water, without any substrate or plants, or on wet towels.²²

In the primary containers, Caudata, would typically be packed individually or in small groups of the same species and it is generally considered poor practice to pack mixed species in primary containers. The primary containers are then included in secondary containers (e.g. polystyrene boxes that can be reused after cleaning and disinfection), which might contain primary containers with Caudata of different species or it is even possible that Caudata and anurans could be packed into the same secondary container. The number of animals for each shipment may vary depending on the species that are transported (Tracey King, Ornamental Aquatic Trade Association Ltd, personal communication, 2018).

The procedures followed for the transportation of salamanders in small-scale trade among keepers and breeders vary depending on the biology and price of the species, but also on the experience of the sender.

Official data on salamanders' import and intra-EU movements are very limited and concern the import of CITES-listed species only; these data are considered to be only a low percentage of the total volume (for an overview on salamanders' trade, see EFSA, 2017). The lack of quantitative official and harmonised data is also due to the lack of a unique commodity code for identifying salamanders, and amphibians in general (EFSA, 2017; EFSA AHAW Panel, 2017).

Other forms of salamander exchange

The trade of captive salamanders is not performed only by official shops and traders.

For Caudata which are traded (i.e. brought, sold, exchanged) between hobbyists, it is likely that 'trade' in larval Caudata occurs. It is extremely likely that such animals are captive bred by hobbyists and, as such, the hobbyists will know each other, and it could be expected that the provenance of these animals will be known (Tracey King, Ornamental Aquatic Trade Association Ltd, personal communication, 2018). A community of amphibian enthusiasts is active in sharing and trading captive salamanders. This segment of trade is very difficult to quantify as it operates on a 'peer to peer' basis, facilitated by social media, Internet fora and discussion groups.

A simple search on the single social networking service 'Facebook'²³ (on January 2018, search word: 'salamanders') shows over 20 closed groups specialised in captive salamander husbandry and

²⁰ Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97.

²¹ <http://www.iata.org/publications/store/Pages/live-animals-regulations.aspx>

²² <http://www.caudata.org/cc/articles/shipping.shtm>

²³ <https://www.facebook.com/>

trading, with many of them based within the EU. The size of these groups ranges from several hundred up to over 20,000 members.

Another option for non-centralised trade-flow happens at exotic pet trade shows. The number of salamanders transported via the community is large in both individuals and species. The biodiversity of captive traded salamanders is very high; the easily kept species like axolotl (*Ambystoma mexicanum*) or sharp-ribbed salamander (*Pleurodeles waltl*) are the most common. Even rare species, species new to science, or those with restricted distribution, can be obtained through social media or fairs: e.g. *Laotriton laonensis* from northern Laos, zoologically described in 2002 (Stuart and Papenfuss, 2002) is nationally protected but became a popular species with well described husbandry (Bachhausen, 2017); *Paramesotriton guanxiensis*, rare species with small area of occupancy in southern China (IUCN Red List; Huang et al., 1983); or the Mangshan crocodile newt, *Tylosotriton lizhenchangii*, from China described in 2012 (Hou et al., 2012). Some salamanders are bartered between keepers, some common species can be found almost free of charge for beginning keepers, whereas the price for rare, specific species or unusual individuals reaches up to hundreds of euros. The hobbyist trade of salamanders is in general based on licences and certificates of captive bred status depending on the national legislation, which may differ among MSs. Movement of rare and protected species is more difficult to trace. However, attempts to place formal restrictions on trade ban in salamanders would likely cause a large proportion of the community going underground and boosting the illegal trade. The community can easily be reached by the social media on which it operates. If support to Bsal prevention is easily available, most salamander keepers are likely to be motivated to follow the guidelines to safeguard their captive animals.

3.2.3.3. Risk-mitigating measures that could potentially be effective in ensuring safer international or intra-EU trade of live salamanders

The list of mitigation measures considered relevant for international and intra-EU movements of live salamanders described by EFSA (2017) has been revised on the basis of new available knowledge, and on the feasibility and effectiveness of the measures (see Section 2.2.4).

In the context of this scientific opinion, the risk-mitigation measures that have been taken into account are the measures that can be applied pre-emptively. In addition, it has to be highlighted that the expert assessment refers only to compliant behaviours.

The possible risk-mitigation measures that could potentially be effective in reducing Bsal risk in international or intra-EU trade of live salamanders, as identified by the experts, are listed and described below (see also Table 3).

The following measures prevent introduction of Bsal by increasing stringency:

- Preventative treatment:

Treating salamanders preventatively before moving is a measure that the experts consider relevant for heat treatment (see Section 3.2.1.2), whereas the preventative use of antimicrobials (antibiotics and antifungals) in salamanders, which have not been confirmed to be Bsal infected, was not considered justifiable. Feasibility of heat treatment was scored from 3 to 5, only if applied to heat-tolerant species of salamanders (e.g. *Triturus dobrogicus*, *Ommatotriton vittatus*, *Salamandra immaculata*, *Cynops orientalis*, *Paramesotriton hongkongensis* and *Salamandra salamandra*). For these species, the effectiveness was scored from 4.5 to 5. An additional aspect highlighted by the experts is that heat treatment can be applied to groups of salamanders and not only individually. Experts noted that heat tolerance has not been assessed across all salamander species.

- Quarantine²⁴:

Quarantine consists of keeping traded salamanders in tanks at the entrance point for 6 weeks, which is the whole Bsal incubation time for *Salamandra salamandra*. Testing the animals for Bsal (in accordance with the protocol reported in Section 3.1.2.1) should take place over the last week of the quarantine. The following actions (release to the destination or treatment of the animals) will be put in place on the basis of the results of the test. Quarantine is reported as a relevant management action (Langwig et al., 2015). However, it requires that proper procedures and facilities are in place at each entry point. The experts scored 4 on the feasibility of this measure, which may vary on the basis of the traded volume, and from 3.5 to 5 on its effectiveness. Comparable quarantine measure should be applied also for intra-EU movements if the animals come from facilities that are not recognised as Bsal-free (see Section 3.1.2.2).

²⁴ In the meantime, Commission Implementing Decision 2018/320 has been published, describing quarantine rules.

- Premovement health certification:

Salamanders showing Bsal clinical signs (skin lesions and ulcers) should not be transported. A premovement health certification²⁵ should report that the salamanders have been individually tested for Bsal (according to the protocol reported in Section 3.1.2.1) before being transported. Animals that are Bsal positive should be properly treated (see Section 3.2.1.2). The validity of the health certificate is commonly specified to 10 days; during this period of time the animals should not be in contact with other amphibians. The experts scored the feasibility of this measure from 3 to 5 and its effectiveness from 4 to 5, taking into account the high variability of the place of origin of the salamanders.

- Bsal testing at the entry point:

This consists of keeping traded salamanders in tanks at the entrance point for one week only, which is the approximate time needed to carry out the Bsal testing (protocol in Section 3.1.2.1). Bsal-positive animals should then be treated according to protocols explained in Section 3.2.1.1. Ideally, all salamanders already kept in captivity in the EU should be Bsal negative (which can be achieved by large-scale screening and treatment, see Section 3.1.2.2) and, therefore, ready to be moved intra-EU. In addition, all live salamanders entering the EU should be tested. The experts scored from 3 to 5 on both the feasibility and the effectiveness of this measure, noting that it does not take into consideration the possible Bsal incubation time which may vary for different species.

- Restriction on salamander importation:

This measure has been set up in the USA in 2016, for 201 species of salamander²⁶ and in Hungary in 2017, for any species of the families Hynobiidae and Salamandridae, and for individuals of the species *Karsenia koreana* (pertaining to the family Plethodontidae).²⁷ The restriction on salamander introduction on the basis of their origin (e.g. from countries where presence of Bsal has been proven) or for some salamander families is a possibility that the experts judged highly feasible (5) and effective (from 4 to 5); however, a clear definition of the restriction criteria and parameters (which countries, which families, which species) needs to be based on progress in the knowledge on Bsal.

- Ban on the importation of salamanders:

A ban on salamander movements at the level of taxonomic order has been considered in EFSA (2017) as depending on the import volumes. In the context of this scientific opinion, this measure has been judged by the experts as a feasible (5) and effective (from 4 to 5) way to prevent introducing Bsal in new areas due to movements of salamanders. However, it needs to be considered that a ban on salamander movements into the EU or intra-EU may boost illegal trade.

The following measures prevent introduction of Bsal when the animals are moved in the EU (irrespective if they originate from a third Country or an EU MS):

- Tracking all traded species:

Current trade records are not complete for species and other import/intra-EU movement data. Therefore, it is considered important to implement a harmonised system for identifying a commodity code for the amphibians (for each shipment) and for tracing the animals that are moved. The experts judged it as a feasible measure (from 4 to 5). Due to the fact that it is related to the host and not to the pathogen, its effectiveness in preventing the spread of Bsal was considered low (score from 1 to 2); however, it was recognised to be useful in tracing back other possibly infected animals.

- Hygiene procedures:

To minimise the potential of Bsal spread, appropriate cleaning and disinfection (see Section 3.2.1.1) of equipment that enters into contact with salamanders (e.g. transport containers, manipulation equipment or boxes for trade) should be applied, before loading the animals, reuse and disposal.

²⁵ In the meantime, Commission Implementing Decision 2018/320 has been published, describing the health certificate.

²⁶ US Federal Register/Vol. 81 No. 8, 13 January 2016 – Rules and Regulations, Fish and Wildlife Service (50 CFR Part16); available at: <https://www.fws.gov/policy/library/2016/2016-00452.pdf>

²⁷ Hungarian Government Decree 199/2017: 199/2017. (VII. 10.) Korm. Rendelet a kedvtelésből tartott állatok tartásáról és forgalmazásáról szóló 41/2010. (II. 26.) Korm. rendelet és a veszélyeztetett vadon élő állat- és növényfajok nemzetközi kereskedelmét szabályozó nemzetközi és európai közösségi jogi aktusok végrehajtásának egyes szabályairól szóló 292/2008. (XII. 10.) Korm. rendelet módosításáról; available at: <https://net.jogtar.hu/jogszabaly?docid=A1700199.KOR×hift=ffffff4&txtreferer=00000001.TXT>

Commodities that are traded with the animals (e.g. water, substrate, soil, plants) should be cleaned and disinfected or safely disposed of. This measure was scored from 4 to 5 in feasibility and 5 in effectiveness.

- Good practice manuals:

The application of good practices was considered relevant in preventing the introduction of Bsal and, therefore, ensuring safe movements of salamanders. This measure consists of setting up a good practice code containing guidelines that should be followed by traders (e.g. the use of cleaned and disinfected boxes, plastic bags for transporting the animals, the use of disposable gloves when handling individual batches, disinfect the water before disposal, if there is organic substrate to incinerate it). The feasibility of this measure was judged 5 and its effectiveness from 4 to 5.

- Application of probiotics to salamanders:

This measure consists of applying microbiota on the skin of the salamanders that are transported. Evidence on the use of probiotics has been reported from laboratory studies, but so far it has not been demonstrated under field conditions. As the information on the use of any probiotic to control Bsal infection is limited, the experts considered this option to be of low feasibility (1–2) and effectiveness (1–2).

In addition, 'vaccination of salamanders' has also been discussed by the experts. In principle, this measure would consist in immunising salamanders with Bsal or related antigens to stimulate a protective response. However, no vaccines have been developed for amphibians (not even for Bd in anurans) and it is still not clear whether induction of a protective immune response in amphibians occurs. Even if studies have been carried out, no immune reaction has been demonstrated in salamanders for Bsal: repeated infection–treatment cycles in Caudata did not induce any significant protection against Bsal infection (Stegen et al., 2017). Immune dampening by Bsal has been reported in a recent publication as highly likely (Farrer et al., 2017). For these reasons the experts excluded this option from the possible risk-mitigation measures.

The best way to ensure safe salamander movements is considered to be a combination of several of the above-mentioned possible measures, for instance import or movement restrictions, entry tests and good practice. In addition to their expected effect in Bsal risk mitigation, other pathogens (e.g. viruses) are still spreading and some may currently be excluded by geographic barriers. Hence, biosecurity still has the potential to mitigate spread of undiscovered and unpredictable pathogens of wildlife (Berger et al., 2016; Richgels et al., 2016).

In conclusion, according to the experts' judgement, banning the import of salamanders or restricting the ban to specific families/genera/species or to specific countries of origin (i.e. where Bsal has been proven) and quarantine (plus Bsal testing) are considered possible measures for ensuring safer international movements. However, their feasibility and effectiveness depend on the volumes of animals that are transported. Banning/restricting trade might also boost illegal movements. An alternative that the experts consider likely to minimise the risk of spreading disease, consists of a combination of best practices and hygiene procedures that minimise importation and intra-EU movements of infected animals. In addition, movements only from captive bred stocks for which the health status is known ('close populations'), should be fostered (see also Section 3.1.2.2).

In Table 3, the risk-mitigation measures identified by the experts to ensure safer international or intra-EU movements of live salamanders have been listed, together with a short description/explanation and reference. The results of the experts' qualitative assessments of their feasibility and effectiveness have been also reported (for the details on the methodology and a full description of the results, see Section 2.2.4 and Appendix C (Section C.3)).

Table 3: Risk-mitigation measures that could potentially be effective in ensuring safer international or intra-EU trade of live salamanders as regards the transmission of Bsal, including diagnosis and potential treatment (s) and qualitative assessment of their feasibility and effectiveness (*) – based on Table 7 in EFSA (2017)

Risk-mitigation measures ^(a)	Definition/explanation	Score for feasibility considering also the consequences (e.g. environment)	Score for effectiveness
Preventative heat treatment	To treat animals in an environment at 25°C for 10 days (see Section 3.2.1.2)	3–5	4.5–5
Quarantine	Previously reported in Grant et al. (2016, 2017) Keep traded salamanders in tanks at entrance point for the whole Bsal incubation time while testing them for Bsal (see Section 3.1.2.1) over the last week	4	3.5–5
Require pre-movement health certification	Previously reported in Grant et al. (2016, 2017) Individual Bsal test of salamanders (see Section 3.1.2.1)	3–5	4–5
Bsal testing at the entry point	Keep traded salamanders in tanks at entrance point while individual testing for Bsal (see Section 3.1.2.1) Positive animals will be treated (according to protocols explained in Section 3.2.1.1)	3–5	3–5
Restrict importation of some salamanders	Previously reported in Grant et al. (2016, 2017) Restrictions/partial ban of salamander introduction on the basis of the origin or for some salamander families	5	4–5
Ban all importation of salamanders	Previously reported in Grant et al. (2016, 2017) Ban of all salamanders trade (Martel et al., 2014; Yap et al., 2015; Berger et al., 2016; Richgels et al., 2016)	5	4–5
Tracking all traded species	Use an unique code for identifying salamanders' (or amphibians' in general) shipments (Auliya et al., 2016; EFSA, 2017)	4–5	1–2
Hygiene procedures	Equipment that enters in contact with salamanders should be cleaned and disinfected (according to the protocols reported in Section 3.2.1) (EFSA, 2017)	4–5	5
Good practice manuals	Draw up and distribute a good practice code containing guidelines for traders for handling salamanders and fomites (Murray et al., 2011b)	5	4–5
Apply probiotics to salamanders	Previously reported in Grant et al. (2016, 2017) Preventative probiotic in salamanders' skin. The method has not been drawn up for Bsal Examples of studies on Bd are reviewed in Bletz et al. (2013)	1–2	1–2

(*): The feasibility was assessed on a continuous scale from 'least feasible' to 'most feasible', integrating aspects related to human resources, technical efforts, treatment costs and environmental side-effects and the effectiveness was assessed on a continuous scale from 'no prevention' to 'blocking measure'. After consensus appraisal of the outcomes of the individual judgements (see Appendix C, Section C.3.1) and discussion of particular reasoning, the overall outcome was represented by an interval covered by the central estimates across all experts. The quintiles of value distribution of judgements were converted into a scale from 1 to 5. The broader is the reported interval, the greater is the uncertainty comprised in the judgements.

(a): Grey cells indicate the risk-mitigating measures that the experts considered relevant to be added to the list reported in Table 7 of EFSA (2017).

Figure 5 summarises the feasibility x effectiveness assessment of risk-mitigation measures that could potentially be effective in ensuring safer international or intra-EU trade of live salamanders, revealing their relationship on a feasibility (x-axis) vs effectiveness (y-axis) plane. No measure was considered certainly perfectly feasible and effective. 'Hygiene procedures', 'GP manuals' and 'trade ban

and restrictions' were considered promising on both scales. Other measures were attached with greater uncertainty in either dimension, i.e. 'preventative heat treatment' and 'pre-movement health certification'. Combinations of measures will improve effectiveness; feasibility may not increase due to the combination of multiple efforts in implementation.

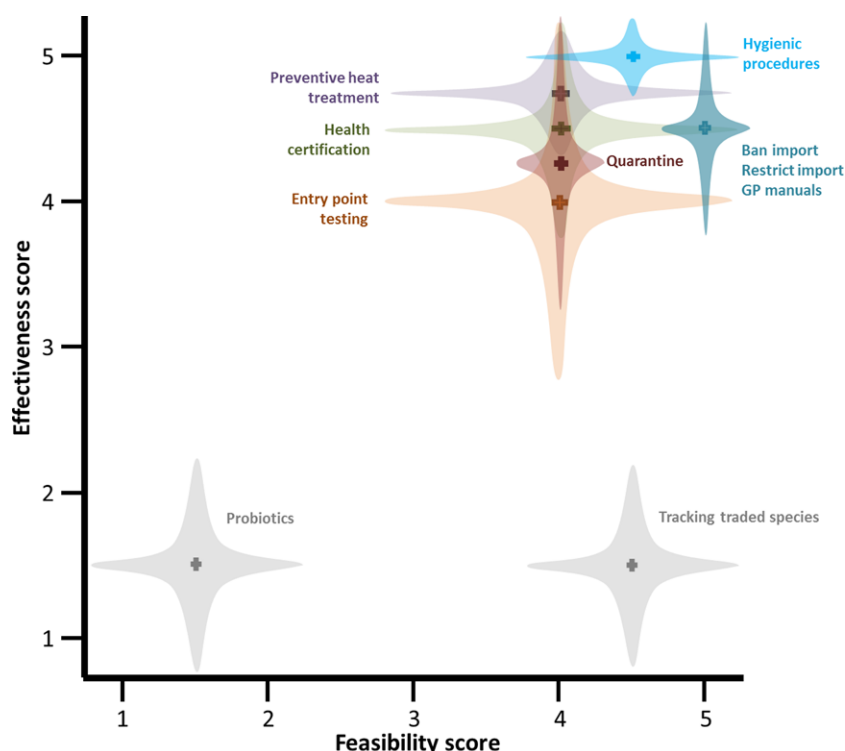


Figure 5: Overview of the consensus judgement and uncertainty for feasibility and effectiveness of the mitigation measures ensuring safer international or intra-EU trade of live salamanders assessed by experts. For exact definition of feasibility and effectiveness used in the judgement and individual level uncertainties see Appendix C (Section C.3). The branch length in each dimension represents the remaining variability in central expert judgement after consensus discussion (see Table 3). The areas under the curves have no meaning

3.2.4. Role of live, silent carriers of Bsal in spreading it as vectors and those of fomites (e.g. waste water, animal by-products, feed) and their risk-mitigating measures (ToR 3c)

For this opinion, the carrier concept is interpreted as any means of spread (biotic and abiotic) of Bsal potentially resulting in transmission to the host. It is differentiated to 'active carriers' (e.g. salamanders, anurans) and 'passive carriers' (e.g. wild birds, water and human activities). Active carriers are hosts acting as biological vectors for the spores, whereas passive carriers are mechanical vectors to which the spores can be attached.

For this ToR, the Bsal-silent carrier concept refers to the passive carriers. Potentially, all the passive carriers of Bsal listed in Table 2 for wild salamanders might be pertinent as silent carriers also for the salamanders in captivity.

Here, only the ones for which evidence has been retrieved are listed and relevant risk-mitigation measures provided.

3.2.4.1. Human movements, activities

Human-driven movements of amphibians and/or Bsal spores: e.g. human movements, or amphibian movements through humans, for conservation or scientific purposes. Zhu et al. (2014), for example, highlighted the need of using disposable latex gloves and to rinse boots and equipment with proper disinfection solution before entering each location, to prevent cross-contamination among sites. In general, defined hygiene procedures and good practices when entering into contact with salamanders and/or their habitats should be followed (see also following Sections 3.3.2 and 3.3.3).

In addition, the release of new animals in a conservation site should be subjected to strict biosecurity rules to prevent any contamination.

3.2.4.2. Water and waste water

Bsal zoospores have been shown to remain infectious in water for at least 31 days at 15°C (Stegen et al., 2017); therefore, to minimise the potential of Bsal spread, appropriate treatment and disinfection (see Section 3.2.1) of the water should be always performed before disposal or flushing down in the canalisation.

The use of tap water is also a safe measure.

3.2.4.3. Substrate

Soil has been shown to remain infective for at least 48 h (EFSA AHAW Panel, 2017; Stegen et al., 2017); therefore, to minimise the potential of Bsal spread, appropriate treatment and disinfection (see Section 3.2.1) of the substrate should be always performed before disposal.

The use of commercial substrate or pre-treatment of the substrate are also safe viable alternatives.

3.2.4.4. Equipment and fomites

Appropriate cleaning treatment and disinfection (see Section 3.2.1) of equipment and fomites that are in contact with salamanders should be always performed before reusing them.

The use of disposable equipment is also a safe viable alternative.

3.2.4.5. Wild birds

Birds, particularly waterfowl, may carry chytrid spores (Hanlon et al., 2017). Bsal adherence on goose feet has been proven experimentally (Stegen et al., 2017); therefore the role of birds in carrying Bsal spores cannot be excluded.

It is reported (Paul Bakuwel, Ornamental Fish International, personal communication, 2018) that kept salamanders are usually kept indoors; however, if outdoor facilities exist they need to be protected from entrance of animals.

3.2.4.6. Anurans (frogs and toads)

Amphibians traded live or as chilled, unfrozen, unskinned legs are considered as potential carriers of amphibian chytrids (Gratwicke et al., 2010). The knowledge on wild frog species as Bsal carriers is limited to a single Asian species, *Bombina microdeladigitata*, a species that is not used in the food trade (Nguyen et al., 2017).

Live anurans

Some species of anurans have been inoculated with Bsal and placed with salamanders: after experimental exposure to Bsal, midwife toads (*Alytes obstetricans*) showed low intensities of Bsal for several weeks. While the toads showed no sign of disease, their colonisation with the pathogen was sufficient to transmit Bsal to susceptible fire salamanders (Stegen et al., 2017). Recently, wild and traded, imported toads (*Bombina microdeladigitata*), presumably stemming from Vietnam, were found to be positive for Bsal (Nguyen et al., 2017). Based on infection trials, healthy carriers may be common in moderately susceptible European Caudata species as well (Stegen et al., 2017), in which Bsal was demonstrated up to more than 3 months post exposure. As this long-term persistence did not result in protection against re-infection, these species may prove suitable Bsal carriers for long periods of times in infected ecosystems (Stegen et al., 2017).

As reported in Section 3.2.3.2, when amphibians are traded, it is possible that Caudata species and anurans packed into different primary containers could be shipped into the same secondary container (Tracey King, Ornamental Aquatic Trade Association Ltd, personal communication, 2018). However, if the animals are transported following the correct hygiene procedures (see Section 3.2.3.3) and good practices, the risk of contamination should be extremely unlikely (1–5% probability range; see EFSA Guidance, 2018).

In addition, the load of Bsal in frogs is low, frogs are not infected in the skin and no specific test is available with the sensitivity to detect Bsal. A possible presence of Bsal in frog's larvae is unknown and several anuran species have not been Bsal tested. Therefore, the role of anurans in carrying Bsal is still unclear.

Anuran products

Frogs' legs are produced within and outside the EU, and meat for the Goliath frog is produced in Cameroon. The most common amphibian product, frogs' legs, is internationally traded and produced by farming or by collection of amphibians in the wild. Belgium, France, Luxembourg and the Netherlands have been the largest EU importers of frog meat in recent decades (Veith et al., 2000; Warkentin et al., 2009; Altherr et al., 2011). The main exporters of frogs' legs to the EU are from Asia: Indonesia, Malaysia, China, and Vietnam (Warkentin et al., 2009; Altherr et al., 2011). The frogs' legs are most commonly traded skinned (skin removed) and frozen. This way of processing should be sufficient to inactivate chytrid fungi (tested on free-living species related to *Batrachochytrium* genus; Gleason et al., 2008).

Anuran by-products

Frogs' leather, poison of jungle frogs and exudation from the skin of certain frogs used as a painkiller or as a poison are reported as by-products obtained by anurans. However, based on the subjected treatments (heat treated or dried) and the low amount of production with very rare import to the EU, the risk that they carry Bsal is considered negligible.

3.3. Protection from Bsal of kept and wild salamanders (ToR 4)

In general, as disease mitigation during outbreaks is unlikely to be successful, control efforts should focus on preventing disease emergence and transmission between populations. So, this emerging wildlife disease is best controlled through prevention rather than subsequent actions (Schmidt et al., 2017).

3.3.1. Protection of captive salamanders in the places where they are kept

3.3.1.1. Information on how the salamanders are kept in captivity

The recommendations on husbandry of salamanders are as complex as the life histories of different species. Salamanders live in almost all climatic conditions from polar tundra to tropical fully humid forests and have arboreal, terrestrial, fossorial (burrowing) and aquatic forms. Hence, depending on the species, the husbandry approaches vary greatly. In general terms, the aquatic species are kept in aquariums, whereas terrestrial species are normally kept in close facilities (terrariums) (Pasmans et al., 2014).

All salamanders are ectothermic, their body temperature is dependent on the environment; therefore, temperature control is an important part of their husbandry. In general, salamanders live in colder temperatures ranging from 15°C to 20°C, with the exception of tropical species. The facilities for keeping salamanders have to be adapted to the thermal requirements of the kept species, and e.g. air conditioned rooms or unheated underground rooms may be used. Water content in enclosures follows the habitat of the given species, from damp conditions with occasional misting to classic aquaria. The water quality has to be continuously maintained, as salamanders are sensitive to elevated ammonia levels (Harvey Pough, 2007; Association of Zoos and Aquariums, 2012).

All salamander species and their larvae are carnivorous, preferring live prey. Food size depends on the species and age, from minute crustaceans, springtails, fruit flies up to small rodents, mice or fish. Some aquatic species are fed with tadpoles or eggs of easily bred frogs. The food is usually bought in specialised pet-shops, bred by the keepers or in some cases collected from the wild (Pasmans et al., 2014).

The size and construction of enclosures depend on the biological requirements, the keepers' preferences and the purpose of keeping the salamanders, etc. The most common setting is use of glass vivaria mimicking the habitat. For quarantine purposes 'spartan enclosures' with easily cleanable surfaces and disposable substrate are used (Pasmans et al., 2014). Keepers and breeders of larger numbers of individuals build outside artificial ponds and tanks, so the animals have natural climatic conditions. Such settings have to be protected from predators (mammals, birds, snakes) and to avoid the possibility of escape. Ponds and large tanks in general provide better conditions for keeping and breeding the salamanders, but complicate the process of keeping track of the numbers and the health of the kept animals (Harvey Pough, 2007; Association of Zoos and Aquariums, 2012; Pasmans et al., 2014).

3.3.2. Risk-mitigating measures and methods for protection of kept salamanders (ToR 4a)

3.3.2.1. Screening of the kept populations

The owners of the salamander kept populations should test all the animals for Bsal (according to the protocol reported in Section 3.1.2.1). After knowing the status of the salamanders, the new obtained animals should be Bsal tested before introduction into the new collection. Bsal screenings of captive populations have been reported for some EU MSs and in the USA (Sabino-Pinto et al., 2015; Fitzpatrick et al., 2016; Klocke et al., 2017). The feasibility of this measure was scored from 4 to 5, because the screening protocols are already well described and, due to the community of keepers, it is possible to reach most of the kept populations (e.g. via social networks). In addition, the keepers have strong motivations to have their salamanders tested. The expected effectiveness is between 2 and 5. The high uncertainty reflects variation in the possible consequent actions (e.g. treatment or euthanasia of the Bsal-positive collections), or incomplete reach of the existing kept populations.

3.3.2.2. Treatment of the positive cases

All animals that test Bsal-positive should be treated according to the protocol reported in Section 3.2.1 before entering enclosures with the other animals. Based on infection dynamics, at least 3 weeks after treatment, animals should be retested for the presence of Bsal. During this period, animals should be kept in quarantine. This is not purely a protection measure: it is an intervention measure for the infected animals and a prevention measure for preventing spread in the remaining population or introducing the pathogen into wild populations. The experts scored its feasibility from 4 to 5, because the treatment protocols are already well described and tested and easy to be implemented. However, there is uncertainty about the suitability of the existing treatments for many species (e.g. heat treatment). The effectiveness was scored from 4 to 5, because efficacy has been proven under laboratory conditions, but still several unknowns exist across the species. Both heat treatment and antimicrobial treatment are highly effective for killing the fungus; while host species may be negatively affected, the fungus is still removed.

3.3.2.3. Increase in owners' awareness

The attitude of the stakeholders involved is a key measure for disease control success, and this attitude is highly dependent on their education and the communication provided to them. Public awareness of the potential introduction of Bsal or the signs/symptoms of infection could aid managers in their responses. The stakeholders involved in keeping salamanders are mainly keepers (hobbyists), breeders and stores.

Examples to increase awareness include disease information (how it spreads, which are the signs, how it could be identified and treated, levels of mortality and morbidity, etc.) and also information on good practices (i.e. on how to prevent the spread of the disease) for keepers and stores that can be spread via the Internet, social media, advertisements, movies, flyers or presentations at meetings/conferences on a regular basis. It includes information papers in the stores for the customers. The experts scored the feasibility of this measure as 5 because sharing information within the community of salamander keepers/breeders and recommendations to minimise disease risks are well developed. Effectiveness was scored between 3 and 4. Doubts on ability to effect sustained behavioural change results in substantial uncertainty.

3.3.2.4. Development of good practices and hygiene protocols

The development of good practices manuals and written guidelines on hygiene protocols that would support safer keeping and management of salamanders in captivity, of the equipment in which they are in contact, and of the environment, is considered an important tool for the protection of kept salamanders.

This measure could be applied to the keepers, breeders and stores/pet-shops.

For keepers/breeders and stores/pet-shops, the experts scored this measure similarly for feasibility (5; few barriers to developing protocols to a high standard) and effectiveness (from 3 to 4). Uncertainty exists about its effectiveness due to issues of compliance and effecting sustained behavioural change.

This measure includes willingness to participate in clean trade practices (absence of Bsal in kept salamanders) and seeking veterinary health management of the kept salamanders (e.g. proper clinical and necropsy procedures).

The manuals should also explain for example: (i) how to handle salamanders, (ii) how to clean and disinfect (or dispose of) the equipment in contact with the animals, (iii) the correct use of water (e.g. tap water), substrate (e.g. commercial) and soil, (iv) the correct disposal of the waste water and substrate (see Section 3.2.4). Information should be given also on animal escape control, e.g. to have equipment for outdoor and indoor housing for preventing the escape of salamanders and the entry of other animals (e.g. birds, rodents). It is also important that the animals are not mixed. Upon acquisition of one or more new animals, they should be Bsal tested and kept separately from the others until the test results are available. The animals that are Bsal positive need to be treated (see Section 3.2.1) and cannot be in contact with the other animals until they test Bsal negative. Salamanders kept in captivity must not be released into the wild nor have direct or indirect contact with the natural environment.

Registration of the facilities where salamanders are kept

This measure is considered relevant because it can give important information on the number of salamanders in captivity, the main families that are kept and the location of the husbandry systems.

It can incorporate keepers, breeders and stores and it implies the presence of an official register and the information may be used e.g. for Bsal outbreaks.

3.3.2.5. Registration of the keepers/breeders of salamanders

Registration of keepers' and breeders' facilities with an indication of at least: (i) where they are located, (ii) the species that are kept (or if species level identification is not possible then at least for the main families kept), and (iii) the range of number of animals that are kept (e.g. 1–10; 10–100; > 100).

As the feasibility of individual registration is influenced by several factors (e.g. the measure is in principle well established but there could be potential reluctance to register if, for example, registration is perceived to be a prescriptive/onerous duty or requirement), the experts scored the feasibility of this measure with high variability, from 1 to 5. This was in contrast with the higher rated feasibility for screening because experts considered that in that case there would be increased individual motivation among keepers for testing their captive population. The effectiveness of registration was scored between 1 and 4 because, although registration should imply recognition of good practice, registration itself is not a measure protective to Bsal spread but rather needs to be combined with further actions.

Registration of the stores/pet-shops of salamanders

Register of the stores and pet-shops selling salamanders with indication of at least: (i) where they are located, (ii) the main species that they sell (or if species level identification is not possible then at least at a family level), and (iii) the maximum number of salamanders that their facilities can keep.

The feasibility of registration of stores/pet-shops was highly evaluated (5), because they are relatively easy to register or are even already registered by law in several MSs.

The effectiveness was scored between 1 and 4 because although registration should imply recognition of good practice, registration itself is not a measure protective to Bsal spread, which needs further actions. In addition, it has to be considered that other forms of salamander exchange exist (see Section 3.2.3.2).

3.3.2.6. Education and training of stakeholders

Considering that it is important that good practices manuals and hygiene procedures are followed by keepers, breeders and stores/pet-shops that deal with salamanders, this measure concerns the organisation of ad hoc training courses on correct keeping and managing amphibians for salamanders' stakeholders. The experts scored the feasibility of this measure from 3 to 4, because it is resource demanding and recruiting stakeholders to attend is likely to be challenging. The effectiveness was scored from 4 to 5 because, providing attendance, practical engagement should enhance good practice.

Table 4 summarises the risk-mitigation measures identified to be relevant for protecting captive salamanders in the places where they are kept, identifies whether each measure is currently in place, given the current knowledge, and reports the results of the experts' opinion assessment on the feasibility and effectiveness of each measure (for the details on the methodology and full description of the results, see Section 2.2.4 and Appendix C (Section C.4)).

Table 4: Risk-mitigation measures and methods for protection of kept salamanders and qualitative assessment of their feasibility and effectiveness (*)

#	Measures	Definition/explanation	In place (e.g.)	Feasibility	Effectiveness
1	Screening of the kept populations	Test skin swabs from captive kept salamanders for presence of Bsal (see Section 3.1.2.1)	Non-systematic screening: BE (research funding) CZ (research funding) DE (research funding) NL (state funding) UK (research funding)	4–5	2–5
2	Treatment of the positive cases	Treatment of the Bsal-positive salamanders (see Section 3.2.1)	BE (private funding) NL (research funding, private funding) DE (research funding, private funding)	4–5	4–5
3	Increase in owners' awareness	Disease information and information on good practices for keepers, breeders and stores via multiple tools (e.g. Internet, advertisements, oral presentations at meetings, socials)	BE, ES, FR, DE, IT, NL, UK	5	3–4
4	Good practices and hygiene protocols for keepers and breeders	Develop good practices manuals and written guidelines with hygiene protocols for correct management of salamanders in captivity, the equipment they are in contact with and the environment For keepers and breeders of salamanders	To a certain extent in: BE, ES, FR, DE, IT, NL, UK	5	3–4
5	Good practices and hygiene protocols for the stores/pet-shops	As above, for the pet-shops and stores selling salamanders	No	5	3–4
6	Registration of the keepers/breeders of salamanders	Registration of keepers' and breeders' facilities with an indication of at least: (i) where keepers are located, (ii) the species that are kept (at least for the main families kept), and (iii) the range of number of animals that are kept (e.g. 1–10; 10–100; > 100)	No	1–5	1–4
7	Registration of the stores/pet-shops of salamanders	Registration of the stores and pet-shops selling salamanders with indication of at least: (i) where they are located, (ii) the main species that they sell (at least at a family level), (iii) the max amount of salamanders that their facilities can keep	In some EU MSs (e.g. IT)	5	1–4

#	Measures	Definition/explanation	In place (e.g.)	Feasibility	Effectiveness
8	Education and training of stakeholders	Organise training courses on correct keeping and managing amphibians for keepers, breeders, stores, pet-shops	No	3–4	4–5

(*): The feasibility was assessed on a continuous scale from 'least feasible' to 'most feasible' and the effectiveness was assessed on a continuous scale from 'negligible' to 'fully protective'. After consensus appraisal of the outcomes of the individual judgements (see Appendix C, Section C.4.1) and discussion of particular reasoning, the overall outcome was represented by an interval covered by the central estimates across all experts. The quintiles of value distribution of judgements were converted into a scale from 1 to 5. The broader is the reported interval, the greater is the uncertainty comprised in the judgements.

Figure 6 summarises the feasibility x effectiveness assessment of mitigation measures and methods for protection of kept salamanders revealing their relation on a feasibility (x-axis) vs effectiveness (y-axis) plane. No measure was considered certainly perfectly feasible and effective. 'Treatment of the Bsal positives', as well as 'good practice/hygiene protocols' and 'increase awareness activities' were measures considered promising on both scales. Other measures were attached with greater uncertainty in either dimension, i.e. 'screening of the kept salamanders'. Combinations of measures will improve effectiveness; feasibility may not increase due to a combination of multiple efforts in implementation.

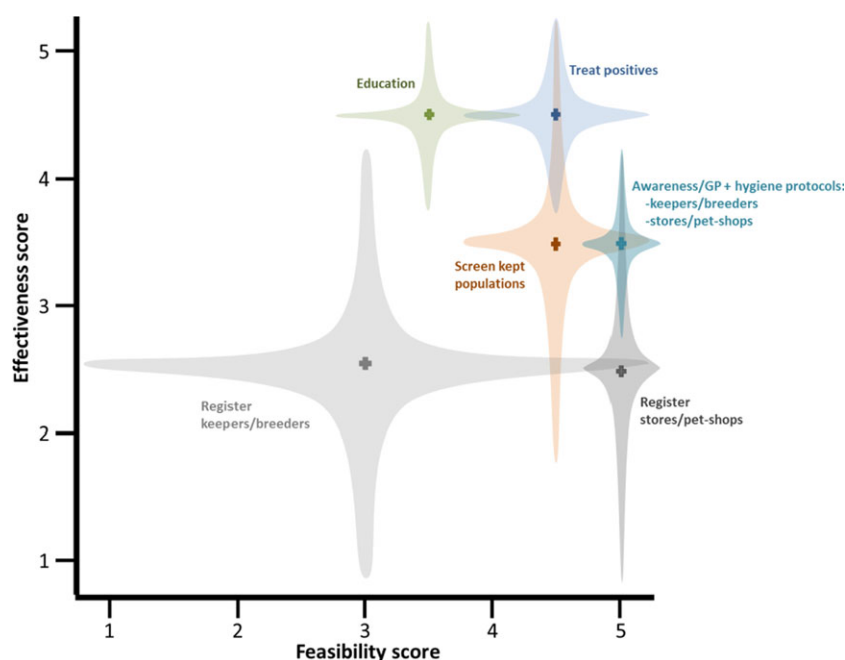


Figure 6: Overview of the consensus judgement and uncertainty for feasibility and effectiveness of the mitigation measures assessed by experts for protection of kept salamanders. For exact definition of feasibility and effectiveness used in the judgement and individual level uncertainties, see Appendix C (Section C.4). The branch length in each dimension represents the remaining variability in central expert judgement after consensus discussion (see Table 4). The areas under the curves have no meaning

3.3.3. Risk-mitigating measures and methods for on-site protection of wild salamanders in their natural habitats (ToR 4b)

There are numerous potential measures and methods that, either alone or when combined, could be considered risk-mitigation actions for the on-site protection of wild salamanders in natural habitats. This section describes a suite of such actions, ranging from direct risk-reduction approaches (e.g. implementing hygiene protocols, such as disinfection) or supporting actions that would increase

feasibility or effectiveness of other actions (e.g. steps that would facilitate early detection of mortality events, such as public awareness campaigns).

The Guidance on the hygiene procedures for the control of diseases in wild amphibian populations (Murray et al., 2011a) was consulted to help set out the measures to address this ToR.

The measures reported below (from Sections 3.3.3.1 to 3.3.3.7) were identified by the experts as the main issues for on-site protection of wild salamanders preventing Bsal introduction and/or spread in their natural habitats. These measures have been assessed for their feasibility and effectiveness (see Table 5).

Phillott et al. (2010) recommended that field researchers evaluate their activities to determine the relative risk of pathogen transmission and spread compared with background levels (i.e. the risk posed by other mechanisms of disease transmission or pathogen dispersal) and implement appropriate strategies to minimise this risk during field studies. The risk of transmission and spread should also be evaluated by researchers, animal ethics committees and government agencies issuing permits and for all other stakeholders working with wild amphibians (e.g. environmental consultants).

Individuals studying amphibians often travel and collect samples of animals from multiple sites. It is important to recognise that humans may aid in the transmission (passing of disease from an infected to an uninfected individual), and spread (movement of disease geographically) of diseases. The risk of disease transmission and spread may be increased due to movement of amphibians or personnel (between isolated areas of habitats or between captive husbandry and laboratory facilities) and by the handling of amphibians.

3.3.3.1. Site definition and visitation

Before commencement of any field work, field sites should be defined robustly to identify potential risks for the spread of disease between sites (see Section 3.1.2.2). The definition of the boundary of a site may not be straightforward and may depend on a number of factors, including habitat characteristics, natural barriers to dispersal and population/meta-population characteristics (see Murray et al., 2011a, for further guidance).

Successively, a strategy for visiting sites should be defined taking into account that multiple sites might be visited in sequence and infection status may differ between sites. In principle, visiting infected sites after uninfected sites should reduce risk of disease spread.

The feasibility of both measures ('site definition' and 'site visitation strategy') was scored by the experts to be 5 for adequately trained/knowledgeable biologists. Experts suggested that for other field workers feasibility might be lower, requiring further training about site definition relevant to wildlife disease.

The effectiveness of 'site definition' was scored from 2 to 3 because it is not a protection measure per se but it must rather be considered as a component of a coherent, multi-action strategy.

The effectiveness of 'site visitation strategy' was rated from 3 to 4. As for the previous measure, defining a site visitation strategy was considered to be of most value as part of a coherent, multi-action strategy, but it was scored to be somewhat more effective than the 'site definition' measure for the prevention of Bsal spread as it represents a direct risk-reduction measure. Experts noted that the feasibility of this measure would be low or not applicable if Bsal status of individual sites is unknown.

3.3.3.2. Hygiene procedures

Cleaning and disinfection of field worker body parts and equipment

Before a site is entered and/or after finishing work at a site, exposed body parts (e.g. hands, arms, knees), clothes and footwear and equipment should be cleaned and disinfected (see Section 3.2.1 for disinfection protocols and Murray et al., 2011a for additional practical details).

Both measures on cleaning and disinfection activities were rated as highly feasible ('cleaning and disinfection of field worker body parts' = 5, 'cleaning and disinfection of field equipment' = from 4 to 5) as the guidelines are already used in practice. Uncertainty relates to the practicality of disinfecting large equipment (e.g. vehicles).

Effectiveness was rated from 3 to 4, for 'cleaning and disinfection of field worker body parts' and from 4 to 5, for 'clean and disinfection of field equipment'. For equipment, more aggressive disinfectants can be used, which was considered likely to raise efficacy. Uncertainty was related to people's willingness to comply with existing guidelines and whether the measures could be consistently performed correctly.

Hygiene procedure and good practices for the movement and handling of wild salamanders

When handling is essential, care must be taken to ensure individuals do not have their exposure to pathogens elevated over background exposure levels.

When possible and practical, disposable items (e.g. single-use latex, nitrile or vinyl gloves, single-use plastic bags, containers) should be used when handling amphibians.

In situations in which gloves are not available or suitable: hand washing with 70% ethanol (allowing hands to dry) between handling individual animals is acceptable (although repeated use on the human skin is not recommended, and because alcohol is toxic to amphibians hands must be washed thoroughly in water after treatment with alcohol). If 70% ethanol is not available, the minimum treatment is hand washing in the water to which the amphibian is normally exposed before and after handling.

In situations in which amphibians must be held temporarily, individuals should be housed in single-use containers (e.g. plastic bags) or in containers disinfected (see above and Section 3.2.1) between each animal, and adults should not be held in groups.

The feasibility of all these four measures ('use of disposable items', 'care in handling', 'hands disinfection', and 'single use of containers') was rated 5, as they are easy to implement and existing protocols for minimising disease risks in amphibians could be easily adapted or applied for Bsal. In addition, handling is generally carried out by experienced people who likely already have some knowledge/training relevant to amphibian diseases or have completed permit applications that should include such considerations.

Effectiveness ratings varied from 2 to 5 ('use of disposable items' and 'single use of containers': from 3 to 4; 'care in handling': from 3 to 5; 'hands disinfection': from 2 to 4). Uncertainty related to people's willingness to comply, whether the measures could be consistently performed correctly, and the extent to which these measures are likely to influence disease transmission within infected sites (i.e. anthropogenic vs natural processes).

Integrated hygiene approach

This measure consists in an integrated approach to prevent or limit disease transmission/spread in wild or wild caught amphibians and it includes the hygiene practices (listed above). Feasibility was scored from 4 to 5, slightly lower than most of the individual measures, as an integrated approach was considered more difficult to implement correctly due to the larger number of steps involved and because some of the feasibility issues of the individual steps remain. However, effectiveness was scored from 4 to 5, higher than most single measures, as a combination of measures is likely to increase the overall effectiveness.

3.3.3.3. Capture and translocation of wild salamanders

Capture, handling and housing of wild salamanders should be minimised or avoided when possible. Translocated amphibians should be treated as if they are infected and should not be transported anywhere for release to the wild. If wild amphibians and their larvae must be housed for some time in a captive situation (e.g. in laboratory, zoo or captive breeding facility), they should not be returned to the wild. This group of measures also includes increasing awareness of practitioners who may capture or translocate salamanders in the wild.

Feasibility of 'avoid capture and handling of salamanders' was scored from 4 to 5 because: it is straight forward to define and this requirement could be reinforced through permit approvals. Effectiveness was scored widely from 1 to 5. Uncertainty for effectiveness related to compliance issues and also awareness of the researchers and other people authorised to work in the field. In addition, there are no procedures currently in place to provide guidance on re-housing or disposing of captured amphibians within the EU.

Both feasibility and effectiveness of 'prevent translocation of wild animals' were scored from 4 to 5, because this measure should be straight forward to define and requirement could be reinforced through permit approvals. Uncertainty in the feasibility was due to the possibility that different field researchers may have different study purposes/priorities (e.g. environmental consultants vs academic research/conservation priorities vs disease-specific priorities).

The experts assessed both feasibility and effectiveness of 'prevent return of the captive animals to wild' as 5; again because this measure is a simple requirement to impose on anyone with a permit to take wild amphibians into captive situations and because it has the potential to completely remove the risk of Bsal spread. Uncertainty for effectiveness was again related to compliance issues.

3.3.3.4. Increase public awareness and participation

The attitude towards risk-reducing practices of stakeholders involved in amphibian-related activities is a key measure for disease control success, and this attitude is highly dependent on the level of education and awareness and the ability to spread good information via communication tools. Public awareness of the potential introduction of Bsal or the signs/symptoms of infection could aid managers in their responses.

Communication between the general public and government agencies responsible for wildlife disease management can facilitate the early detection of invading pathogens (Langwig et al., 2015).

Examples to increase awareness include education campaigns to promote participation and enforce hygiene standards (see Sections 3.3.2.3 and 3.3.2.4), installation of signage for people visiting natural areas (e.g. forests, parks), and producing informational flyers. It also includes facilitating reporting of signs/symptoms of certain issues (e.g. sightings of invasive species), and for disease related issues, providing a means to communicate observations of sick or dead animals (e.g. via creation of hot-lines).

The experts scored the feasibility of this measure from 4 to 5 because stakeholders are reasonably easy to reach (e.g. via the Internet, signage); however, effectiveness was rated between 3 and 4 because this preventative measure is only based on information dissemination, perceived to have a potentially limited capacity to effect sustained behavioural change. This limitation is also reflected by the uncertainty.

3.3.3.5. Passive surveillance

A reserve capacity of diverse and flexible teams is needed to address wildlife disease outbreaks should biosecurity measures fail and a disease is introduced (Berger et al., 2016). Bsal-passive surveillance involves the collection of dead salamanders, which should then be sent for disease diagnosis. Wildlife disease emergency teams should be set up for passive surveillance, as contact points for reporting and consultation if a dead salamander is found. The role of the contact point is to make sure that the reported animal is investigated for Bsal and discount it if it is not relevant. The emergency team includes a reference laboratory where testing dead salamanders for Bsal can be conducted, reporting the Bsal-positive animals and accompanying relevant information (e.g. the site where the Bsal-positive dead salamander has been found) to the local authorities (see also Section 3.1.2.2).

The feasibility of 'collect and send dead amphibians for Bsal diagnosis' was scored from 4 to 5 because guidelines on submission of dead animals already exist for its implementation. The experts scored the effectiveness of this measure from 3 to 4 because, if implemented, it supports early detection but it is not itself a risk-mitigation measure, although it was agreed that information on Bsal detection could subsequently lead to further targeted risk-reducing measures.

The feasibility of 'set up wildlife disease emergency teams for passive surveillance' was scored from 4 to 5. Uncertainty related to the implementation of this measure in local situations in which monitoring is not yet in place. The effectiveness of this measure was scored as 4 because it depends on submission activities and, as above, subsequent actions available to further mitigate risks.

3.3.3.6. Wild population monitoring

Enhancing the collation and retrieval of data on salamander population sizes, distributions and trends is considered important for conservation management. *In situ* management activities should strive to be working with the best available data on population sizes and conservation status. Population monitoring from clearly defined and harmonised surveys can help provide such information. However, to date, the collection of data on salamanders in the EU is fragmented.

The experts judged the feasibility of this measure as 4. Some uncertainty relates to resource demands for amphibian population monitoring. Effectiveness was scored from 3 to 4, because this measure is not a risk-mitigation measure to prevent the spread of Bsal per se, but rather a supportive measure that can help improve the feasibility and effectiveness of other measures.

3.3.3.7. Active surveillance

Active surveillance is the proactive collection of data on Bsal presence in amphibian populations to promote early detection for disease introduction. This form of surveillance involves visiting subpopulations of salamanders and screening for disease according to a clearly defined and harmonised sampling strategy, with the aim of being able to robustly discriminate disease presence from absence. Developing a robust strategy requires information from a range of domains, from

characteristics of the population being sampled (e.g. host population size and distribution) to diagnostic test properties. For Bsal, much of information that is likely required to develop an effective active surveillance strategy is considered limited (see Section 3.1.2.2). Feasibility was so scored from 2 to 4, with the degree of uncertainty related primarily to the resource demands. Effectiveness was scored as 4 because it depends on the quality of the sampling strategy put in place as well as subsequent actions available to further mitigate risks should Bsal be detected.

Table 5 summarises the risk-mitigation measures for on-site protection of wild salamanders in their natural habitats identified to be relevant to responding to the threat of Bsal in the EU, identifies whether each measure is currently in place, given the current knowledge, and reports the results of the experts' opinion solicitation on the feasibility and effectiveness of each measure (for details on the methodology and full description of the results, see Section 2.2.4 and Appendix C (Section C.5)).

Table 5: Risk-mitigation measures and methods for on-site protection of wild salamanders in their natural habitats and relevant qualitative assessment of feasibility and effectiveness^(*)

#	Measures	Definition/explanation	In place (e.g.)	Feasibility	Effectiveness
1	Site definition	Defining a site robustly to identify opportunities for disease spread between sites	No	5	2–3
2	Site visitation strategy	Defining a site visitation strategy when attending multiple sites in sequence	No	5	3–4
3	Cleaning and disinfection of field worker body parts	Cleaning and washing or wiping with a suitable disinfectant (see Section 3.2.1) of hands, arms, knees, etc. before a site is entered and/or after finishing work at a site	Flanders (BE)	5	3–4
4	Cleaning and disinfection of field equipment	Cleaning and disinfection of footwear and equipment (e.g. vehicles, nets, balances) at the commencement of fieldwork and between each sampling site	Flanders (BE)	4–5	4–5
5	Use disposable items	Use of disposable items (e.g. single-use latex, nitrile or vinyl gloves) for moving and handling salamanders	Flanders (BE)	5	3–4
6	Care in handling salamanders	If handling is essential, ensure that individuals do not have their exposure to pathogens elevated over their background exposure levels	Flanders (BE)	5	3–5
7	Hands disinfection	If gloves for handling animals are unavailable, disinfect hands with 70% ethanol and wash hands thoroughly in water after treatment. If ethanol is not available, the minimum treatment is hand washing in the water to which the amphibian is normally exposed	Flanders (BE)	5	2–4
8	Single use of containers for temporarily holding salamanders	For temporarily held animals, ensure single-use or disinfected containers between each animal. Adults should not be held in groups	No	5	3–5
9	Integrated hygiene approach	An integrated approach to prevent or limit disease transmission includes all the single measures on hygiene procedures and good practices outlined above	Flanders (BE)	4–5	4–5
10	Avoid wild salamanders capture and handling	Avoiding or minimising the capture, handling and housing of wild salamanders helps reduce or remove anthropogenic sources of disease transmission between animals and introduction/spread from one place to another	Flanders (BE)	4–5	1–5
11	Prevent translocation of wild animals	Treating translocated amphibians as if they are infected can help mitigate the risk of intentional translocation and therefore disease spread	Flanders (BE)	4–5	4–5

#	Measures	Definition/explanation	In place (e.g.)	Feasibility	Effectiveness
12	Prevent return of captive animals to the wild	Wild amphibians and their larvae that need to be housed for some time in a captive situation (e.g. laboratory, zoo or captive breeding facilities) should not be returned to the wild. The measure includes increasing awareness of salamanders' keepers that should not release kept salamanders into the wild	No	5	5
13	Increase public awareness and participation	Disease information and education campaigns to promote participation and enforce hygiene protocols and good practices via multiple tools (e.g. installation of signage for people visiting natural areas and producing informational flyers). It includes facilitating reporting of signs/symptoms (e.g. sighting for invasive species) and means to communicate observations of sick or dead animals (e.g. via creation of hot-lines)	AU, BE, FR, DE, IT, ES, NL, UK	4-5	3-4
14	Collect and send dead animals for Bsal diagnosis	As a part of passive surveillance, dead amphibians should be collected and sent for disease diagnosis	Flanders (BE)	4-5	3-4
15	Set up wildlife disease emergency teams for passive surveillance	A contact for reporting and consultation in the case a dead salamander is found. This measure includes a laboratory where testing salamanders for Bsal (see Section 3.1.2.1), reporting the Bsal-positive animals and accompanying relevant information to the local authorities can be conducted (see Section 3.1.2.2)	BE, FR, DE, IT, ES, NL, UK	4-5	4-5
16	Wild population monitoring	To collate and retrieve data on population sizes, distributions and trends	In some MSs (see Section 2.1.2), however no database is available	4	3-4
17	Active surveillance	To actively collect data on Bsal presence in amphibian populations by visiting and screening for disease local populations of salamanders according to a clearly defined and harmonised sampling strategy (see Sections 3.1.2.1 and 3.1.2.2)	In some extend, in some MSs (see Sections 2.2.2.2 and 3.1.1.3)	2-4	4

(*): The feasibility was assessed on a continuous scale from 'least feasible' to 'most feasible' and the effectiveness was assessed on a continuous scale from 'negligible' to 'fully protective'. After consensus appraisal of the outcomes of the individual judgements (see Appendix C, Section C.5.1) and discussion of particular reasoning, the overall outcome was represented by an interval covered by the central estimates across all experts. The quintiles of value distribution of judgements were converted into a scale from 1 to 5. The broader is the reported interval, the greater the uncertainty comprised in the judgements.

Figure 7 summarises the feasibility x effectiveness assessment of risk-mitigation measures and methods for on-site protection of wild salamanders in their natural habitats revealing their relation on a feasibility (x-axis) vs effectiveness (y-axis) plane. Colour coding follows the logical structure of the section above (e.g. green used for all hygiene-related measures). One measure was considered certain perfectly feasible and effective being the 'prevention of return to the wild after temporary captivity'. 'Disease emergency teams', 'prevent translocation of wild animals', 'integrated hygiene approach' and 'cleaning/disinfection of equipment' were considered promising on both scales. Combinations of measures will improve effectiveness; feasibility may not increase due to the combination of multiple efforts in implementation (e.g. compare 'integrated hygiene approach', i.e. the combination, to single hygiene measures all with perfect feasibility but limitations in effectiveness).

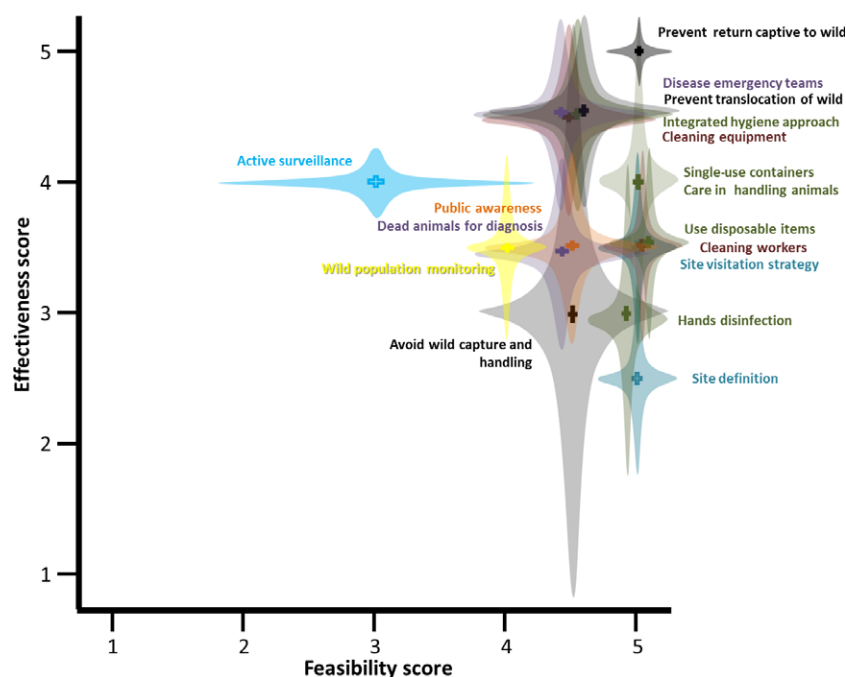


Figure 7: Summary of the consensus judgement and uncertainty for feasibility and effectiveness of the mitigation measures assessed by experts for on-site protection of wild salamanders. For exact definition of feasibility and effectiveness used in the judgement and individual level uncertainties see Appendix C (Section C.5). The branch length in each dimension represents the remaining variability in central expert judgement after consensus discussion (see Table 5). The areas under the curves have no meaning

3.3.3.8. Other aspects to be taken into account when considering protection of salamanders in their natural habitats

Most of the measures described above relate to disease risk-reducing activities related to introduction and further spread of disease, especially those for which some evidence of reasonable feasibility and/or efficacy is already known or there has been proof-of-concept.

However, there are additional measures that although not currently considered feasible/effective could become so in the future given additional investigation or in some cases if a major scientific breakthrough is achieved. There are also some measures that have been trialled for Bd in the wild and have exhibited some promise but only in very specific circumstances (*in situ* treatment, site disinfection; Bosch et al. 2015; Hardy et al., 2015; Hudson et al., 2016; Drawert et al., 2017).

Most measures considered for eradication of Bsal from the wild after it has become established (e.g. on-site treatment, site disinfection) fall into this group of measures, as do measures that may fundamentally alter the host–pathogen relationship in favour of host survival (vaccination, probiotic treatments).

Other measures could also conceivably slow or restrict the natural spread of Bsal from known infected sites but have not been field tested (e.g. barrier fencing, culling, habitat manipulation).

Other measures still are focused on stewarding threatened species through disease epidemics until longer term solutions are proven or discovered (e.g. setting up captive backup populations, selective breeding and population supplementation).

The measures listed in this section have not been assessed by the experts given their limited relevance to responding to the present threat of Bsal introduction and/or spread in the EU.

3.3.3.9. Integrated infection management

The decision process for setting up mitigation strategies requires integrated thinking that assesses mitigation options critically and embeds them within more comprehensive strategies for the conservation of amphibian populations, communities and ecosystems (Garner et al., 2016). Provided proper infection and population monitoring is implemented, combining several mitigation tools in so-called integrated strategies is likely to increase the effectiveness of infection management. Integrated strategies are also

preferred as no single measure is universally applicable. Hence, the capacity to combine several tools is a factor modulating infection management success. However, the success of infection management in wildlife also depends on several other factors: (a) the single or multihost nature and other characteristics of the pathogen, (b) the availability of suitable diagnostic tools, (c) the characteristics of the wildlife host (s), (d) the geographical range of the pathogen/reservoir (improved control in isolated versus continuous populations) and the scale of the intervention effort (large-scale longitudinal programmes are better), and (e) the attitude of the stakeholders involved (Gortázar et al., 2015).

It has also been postulated that transnational responses to infectious disease often come 'too little, too late' to prevent the entry and spread of highly lethal fungal pathogens (Fisher et al., 2016). Hopefully, the tools reviewed above, and in particular their combined use in integrated strategies, will contribute to mitigate the potential risks of Bsal for salamander conservation in Europe.

4. Conclusions

ToR 2b: As regards presence, absence, surveillance and eradication, assess possible identification of various areas (e.g. countries, zones, territories) which may be considered infected with Bsal or free from it

- 1) Bsal surveillance is currently limited: active surveillance in wild salamanders is ad hoc in Austria, Belgium, Croatia, the Czech Republic, France, Germany, Portugal, Slovenia, Spain, Switzerland, the Netherlands and the United Kingdom; passive surveillance in wild salamanders via contact points/emergency teams is temporally in place in Belgium, France, Germany, Spain, the Netherlands and the United Kingdom; and surveillance in captive salamanders is ad hoc in Belgium, the Czech Republic, Germany, Spain, the Netherlands and the United Kingdom.
- 2) Bsal has been detected in collections of captive salamanders in Belgium, Germany, Spain, the Netherlands and the United Kingdom, and in wild populations in some regions of Belgium, Germany and the Netherlands.
- 3) The absence of detection (in particular in the wild) does not mean the absence of Bsal.
- 4) Data on salamander species distribution in the EU are available, meanwhile abundance data are generally not comprehensive.
- 5) According to niche modelling, at least part of the range of distribution of every salamander species in the EU overlaps with the climate conditions predicted to be suitable for Bsal.

ToR 2d: As regards presence, absence, surveillance and eradication, assess suitability of surveillance methods to ensure reliable and robust demonstration of presence or absence of Bsal

- 6) The Bsal-specific qPCR has been shown to have a high specificity and sensitivity being a reliable and feasible diagnostic tool.
- 7) Passive surveillance is considered the most suitable approach for detection of Bsal emergence in wild host populations.
- 8) An active surveillance approach is likely to be both resource and data intensive. Some of the key items to construct an active surveillance are: potential points of entry to the wildlife habitat, species susceptibility, population sizes and knowledge of species distributions. Data on these items are currently lacking.
- 9) Demonstration of Bsal absence in wild populations of salamanders is not currently considered feasible.
- 10) It would be feasible to demonstrate absence of Bsal in a 'closed population' of a susceptible salamander species kept in captivity by sufficiently long quarantine and absence of clinical symptoms of Bsal confirmed by visual observation. This should be complemented by testing all animals at the end of a quarantine period.

ToR 3a–i: As regards spread of Bsal in and from infected areas or via infected animals or fomites, assess the risk of survival, spread and establishment of Bsal within already infected areas and spread from infected areas into other parts of the EU by natural movements of live salamanders taking into account especially relevant geographical, hydrographical and meteorological conditions

- 11) In the wild, Bsal can possibly be spread by both active carriers (e.g. salamanders, anurans) and passive carriers (e.g. wild birds, water). Bsal is most likely maintained and/or spread in infected areas by intra- and interspecies contacts of salamanders but potentially also by interactions with anurans.
- 12) Bsal entry into new areas and populations is most likely to be caused by human activities (mainly amphibian-related), but potentially also by wild birds and mammals and through connected streams of water.

ToR 3b: As regards spread of Bsal in and from infected areas or via infected animals or fomites, assess risk-mitigating measures that could potentially be effective in ensuring safer international or intra-EU trade of live salamanders (both captured in the wild and bred) and their products and by-products as regards the transmission of Bsal including diagnosis and potential treatment(s)

- 13) The risk-mitigation measures that were considered most feasible and effective for live salamanders were either a ban or restrictions on salamander imports, hygiene procedures and good practice manuals.
- 14) The feasibility and effectiveness of a ban or restriction on salamander import and trade depend on the volumes of animals that are currently transported; these measures might also boost illegal movements.
- 15) The effectiveness of heat treatment was considered high. However, the heat tolerance of many salamander species is unknown.
- 16) Treatments with antimicrobials can be justified only for confirmed Bsal-positive animals, when not suitable for heat treatment.
- 17) For salamander products and by-products, heat-processing or desiccation is feasible and effective to ensure safe international and intra-EU trade.
- 18) Combining several risk-mitigation measures (an integrated infection management strategy) will improve overall effectiveness.

ToR 3c: As regards spread of Bsal in and from infected areas or via infected animals or fomites, assess the role of live silent carriers of Bsal in spreading it as vectors and those of fomites (e.g. waste water, animal by-products, feed) and their risk-mitigating measures

- 19) In populations of kept amphibians, Bsal spread can potentially occur via passive carriers such as human movements and activities, waste water, equipment, substrate and fomites. These risks can be mitigated by implementing hygiene procedures and good practices.
- 20) Bsal contamination of traded and/or kept salamanders via live silent carriers (wild birds and anurans) is considered extremely unlikely as salamanders are normally kept indoors and separated from anurans, if good practices are implemented.

ToR 4a: As regards protection from Bsal, assess the potential and feasible risk-mitigating measures and methods in kept salamanders

- 21) The most feasible and effective risk-mitigation measures for protection of kept salamanders from Bsal are the identification and treatment of positive collections.
- 22) Mitigation measures for awareness, good practice and hygiene protocols are considered feasible. Their effectiveness will increase by combining the available measures into an integrated infection management strategy.

ToR 4b: As regards on-site protection from Bsal, assess the risk-mitigating measures and methods for salamanders in their natural habitats.

- 23) The most feasible and effective risk-mitigation measures for on-site protection of wild salamanders from Bsal are: preventing release/return to the wild of kept or temporarily housed wild salamanders and the translocation of wild amphibians.
- 24) Setting up contact points/emergency teams in support of passive surveillance is considered feasible and effective.

- 25) Mitigation measures for hygiene procedures were considered feasible for anthropogenic sources of risk, but less effective for natural mechanisms of spread (e.g. intra- or inter-species transmission, potential carriers such as wild birds). Their effectiveness is likely increased as part of an integrated approach.

5. Recommendations

- 1) Introduction of a harmonised system/protocol for Bsal detection throughout the EU is desirable. Further research is needed to design an efficient surveillance system that accounts for the complexities of additional introduction or spread of Bsal in the EU (e.g. on potential points of entry to the wildlife habitat, species susceptibility to natural infection, population sizes and knowledge of species distributions), and to enable a risk analysis per species.
- 2) Data acquisition on abundance and distribution of salamanders across EU is needed for future risk assessments, surveillance and eventual intervention; such new information would also improve niche modelling for infection of Bsal.
- 3) Passive surveillance activities for Bsal would be enhanced with the implementation of contact points/emergency teams in charge of all aspects of samples collection and management and coordinating laboratory testing and information flow.
- 4) Increasing awareness among professionals and the public of the role of enhanced passive surveillance is recommended to improve early detection.
- 5) Movements of captive animals should be conditional on known health status for Bsal (Bsal test negative).
- 6) The testing of quarantined animals should be performed as close as possible to the end of the quarantine with qPCR (maximum in the last week).
- 7) Further studies are needed on thermotolerance treatments for Bsal as well as infection susceptibility of amphibians (potential hosts, carriers, vectors).
- 8) Information about submitting samples for diagnostic, treatments, hygiene protocols and good practices on Bsal should be provided to salamander keepers.
- 9) Guidelines on hygiene procedures and best practices need to be distributed to all relevant stakeholders and could become integral components of permits for dealing with wild salamanders (site visitation, capture, handling and movement).

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Glossary

Animal by-products	not meant for human consumption
Animal products	meant for human consumption
Bsal-susceptible species	species that can become infected by Bsal and show clinical signs and disease (with different levels of gravity up to death)
Bsal carrier	any means of spread (biotic and abiotic) of Bsal potentially resulting in transmission to the host. Active carriers are hosts acting as biological vectors for the spores, whereas passive carriers are mechanical vectors to which the spores can be attached. A Bsal carrier that cannot be identified is a silent carrier
Bsal-resistant species	species that do not become infected by Bsal
Bsal-tolerant species	species that can become infected by Bsal in the absence of clinical signs and pathology
Close population	population of kept salamanders that has no exchange with others (no immigrations no emigrations). In the context of this opinion, a 'close population' would imply also appropriate implementation of hygiene procedures and good practices
Disease spread	movement of disease geographically
Disease transmission	passing of disease from an infected to an uninfected individual
Meta-populations of salamanders	populations of salamanders separated by space but connected by dispersal of individual salamanders
Newts	representatives of one group within the family Salamandridae (subfamily Pleurodelinae). For taxonomic consistency, the use in this scientific opinion of the term 'salamanders' is inclusive of 'newts'
On-site protection	protection that conventionally refers to wild animals in their natural habitats
Subpopulation of salamanders	group of salamanders that can contact each other. It is equal to 'local population' and 'population on a site'
Synonym	in taxonomy, is a system of accepted alternative names for species

Abbreviations

AHAW	Animal Health and Welfare
AHL	Animal Health Law
AU	Austria
Bd	<i>Batrachochytrium dendrobatidis</i>
BE	Belgium
Bsal	<i>Batrachochytrium salamandrivorans</i>
CI	confidence interval
CITES	Convention on International Trade in Endangered Species

CL	confidence level
CZ	Czech Republic
ddPCR	droplet digital PCR
DE	Germany
ELS	Extensive Literature Search
ES	Spain
FR	France
GE	Genomic Equivalent
IATA	International Air Transport Association
IR	Ireland
IT	Italy
IUCN	International Union for Conservation of Nature
LAR	Live Animal Regulations
MS	Member State
NCA CR	National Conservation Agency in Czech Republic
NGO	Non-Governmental Organisation
NL	the Netherlands
OIE	The World Organisation for Animal Health
PCR	Polymerase Chain Reaction
qPCR	Real-time Polymerase Chain Reaction
RAVON	Reptile, Amphibian and Fish Conservation the Netherlands
sp.	Species
syn.	Synonym
ToRs	Terms of Reference
USA	United States of America
UK	United Kingdom
WG	Working group

Appendix A – Extensive literature search

A.1. Sources of information included in the search

Search strategies were undertaken to identify scientific literature on *Batrachochytrium salamandrivorans*. The following resources were searched to identify relevant studies:

A) Bibliographic databases

- 1) Web of Science (<http://www.webofknowledge.com>), encompassing the following databases (inception – 16 October 2017):
 - Web of Science™ Core Collection
 - BIOSIS Citation Index
 - CABI: CAB Abstracts®
 - Chinese Science Citation DatabaseSM
 - Current Contents Connect®
 - Data Citation IndexSM
 - FSTA® – the food science resource
 - KCI-Korean Journal Database
 - Russian Science Citation Index
 - MEDLINE®
 - SciELO Citation Index
 - Zoological Record®
- 2) Scopus (<https://www.scopus.com>) (inception – 16 October 2017)
- 3) PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>) (inception – 16 October 2017)

B) Search engines

- 1) Invasive Species Compendium (<http://www.cabi.org/isc/>)
- 2) Google Scholar: to facilitate the treatment of the results, Google Scholar were be searched via Publish and Perish (<http://www.harzing.com/resources/publish-or-perish>)
- 3) OpenAIRE (<https://www.openaire.eu/>)
- 4) WorldWideScience (<http://www.worldwidescience.org>): limited to papers and public access.

A.2. Sources of information included in the search

The search strings were designed to retrieve relevant documents to '*Batrachochytrium salamandrivorans*'. The genus name, *Batrachochytrium*, was not included as an independent term in the search strings in an attempt to maximise the precision of the searches, since it will retrieve publications on '*Batrachochytrium dendrobatidis*' that are non-relevant for this scientific opinion.

1) Web of Science (all databases)

Date of the search 16 October 2017

Set	Query	Results
#1	TS=(((Bsal OR Bs) AND (Urodela* OR salamand* OR Newt\$ OR amphibia*)) OR salamandrivorans) Time span=All years Search language=Auto	124

After de-duplication with the 2016 searches results: 37; after removing record from 1948: 36.

2) Scopus

Date of the search 16 October 2017

Set	Query	Results
#1	TITLE-ABS-KEY (((bsal OR bs) AND (urodela* OR salamand* OR newt\$ OR amphibia*)) OR salamandrivorans)	47

After de-duplication with the 2016 searches results: 15; after removing record from 1979: 14.

3) PubMed

Date of the search 16 October 2017

Set	Query	Results
#1	Search (((("Urodela"[Mesh] OR urodela*[tiab] OR salamandr*[tiab] OR salamander*[tiab] OR newt[tiab] OR newts[tiab] OR amphibia*[tiab]) AND (Bsal[tiab] OR Bs[tiab]))) OR salamandrivorans[tiab]	29

After de-duplication with the 2016 searches results: 9.

A.2.1. Search strings used in search engines1) Invasive Species Compendium. Advanced bibliographic search (<http://www.cabi.org/isc/>)

Date of the search 16 October 2017

Query	Results
In all fields: ((((((Bsal OR Bs) AND (urodela* OR salamander* OR salamandr* OR newt* OR amphibia*)))) OR (salamandrivorans))	10

After de-duplication with 2016 searches and within the resource: 2.

2) Google Scholar (via Publish and Perish)

Date of the search 26 October 2017

Query	Results
All the words: Salamandrivorans	450

After de-duplication with 2016 searches and within the resource: 235.

3) OpenAIRE (<https://www.openaire.eu/>)

Date of the search 26 October 2017

Query	Results
Salamandrivorans	27

After de-duplication with 2016 searches and within the resource: 6.

4) WorldWideScience (<http://www.worldwidescience.org>)²⁸

Date of the search 26 October 2017

Query	Results
Salamandrivorans Limited to papers and public access documents	270

After de-duplication with 2016 searches and within the resource: 30.

A.3. Refinement of literature search results

The number of results retrieved from each information source was recorded. The output of the searches, i.e. records retrieved from bibliographic databases and grey literature, was exported to EndNote x8 together with the relevant metadata (e.g. title, authors, abstract).

In total, 957 records resulted from the initial searches and were exported to an EndNote library. Duplicates among the 2016 ELS and records dated before 2013 were first removed; the 332 resulting records were compared and removed when two or more records were identical within the same resource and/or across all resources (i.e. author/s, title, journal, pages, doi number). This yielded 250 records which were distributed in three EndNote libraries/Microsoft Excel files:

- 1) Bsal bibliographic database, including the results for Web of Science (all databases), PubMed and Scopus and after de-duplication and removing of records dated before 2013: 41 records.

²⁸ The results of WorldWideScience were limited to papers and public access documents.

- 2) Bsal search engines, including the results for Google scholar OpenAIRE, WorldWideScience, and Invasive Species Compendium and after de-duplication and removing of records dated before 2013: 209 records.

Titles and abstracts were screened for relevance and to remove additional duplicates. The screening was performed by two reviewers in parallel. Records were excluded if considered as duplicates or not relevant and 47 records (43 papers and 4 supporting publications) were considered pertinent after consensus reached by the two reviewers.

Screening of full-text publications, limited to publications in English, was carried out by ad hoc experts when title and abstract did not allow assessing the relevance of a paper, and some of the publications were not considered relevant (resulting in a final number of 26 relevant papers and 4 supporting publications) or proving any additional value to address the question.

An overview of the numbers of the records that resulted from each step of the ELS is reported in Tables A.1, and A.2 shows the list of relevant publications resulting from the ELS.

Table A.1: Overview of the number of results of the ELS

Initial search	Database		Initial count	Post de-duplication among the 2016 ELS results and removing of records dated before 2013
16/10/2017	WoS (All databases)		124	36
16/10/2017	Scopus		47	14
16/10/2017	PubMed		29	9
16/10/2017	Invasive Species Compendium. Advanced bibliographic search		10	2
26/10/2017	Google Scholar		450	234
26/10/2017	OpenAIRE		27	6
26/10/2017	WorldWideScience		270	30
		Total	957	332
				Number of records
After de-duplication within the same resource results and among all the resources				250
After screening of titles and abstracts to identify additional duplicates and relevant literature				43
After full texts screening, limited to English publications, to identify relevant literature				26

Table A.2: List of relevant publications resulting from the ELS

ID	Reference
1	Bachhausen (2017)
2	Dillon et al. (2017)
3	DiRenzo et al. (2017)
4	Drawert et al. (2017)
5	EFSA (2017)
6	Farrer et al. (2017)
7	Feldmeier et al. (2016)
8	Fisher et al. (2016)
9	Fisher (2017)
10	Garner et al. (2016)
11	Geiger et al. (2017)
12	Grant et al. (2017)
13	Hanlon et al. (2017)
14	Klocke et al. (2017)
15	Laking et al. (2017)

ID	Reference
16	Liew et al. (2017)
17	Nguyen et al. (2017)
18	Parrot et al. (2017)
19	Rohr et al. (2017)
20	Schmidt et al. (2017)
21	Speybroeck and Steenhoudt (2017)
22	Stegen et al. (2017)
23	Valenzuela-Sanchez et al. (2017)
24	Van Rooij et al. (2017)
25	Wang et al. (2017)
26	White et al. (2016)

Appendix B – Details of the surveillance activities in place in the wild in some European countries

Country	Region/ areas	Year	Species tested	Number of animals tested	Number of positive animals	Number of negative animals	Published/ acknowledged(*)
Austria	Turnsee lake	2017	<i>Ichthyosaura alpestris</i>	13		13	(1)
Austria	Vienna	2017	<i>Salamandra salamandra</i>	187		187	(2)
Austria	Carynthia	2017	<i>Salamandra salamandra</i>	55		55	(2)
Austria	Salzburg	2017	<i>Salamandra salamandra</i>	7		54	(2)
Austria	Tyrol	2017	<i>Lissotriton vulgaris</i>	2		2	(2)
Austria	Tyrol	2017	<i>Ichthyosaura alpestris</i>	100		100	(2)
Austria	Tyrol	2017	<i>Salamandra salamandra</i>	43		43	(2)
Austria	Tyrol	2017	<i>Triturus cristatus</i>	2		2	(2)
Austria	Tyrol	2017	<i>Salamandra atra</i>	15		15	(2)
Austria	Vorarlberg	2017	<i>Salamandra atra</i>	80		80	(2)
Belgium	Liège	2013	<i>Salamandra salamandra</i>	2	2		Spitzen-van der Sluijs et al. (2016)
Belgium	Vlaams Brabant	2014	<i>Salamandra salamandra</i>	1		1	(3)
Belgium	Liège	2014	<i>Salamandra salamandra</i>	35	35		Spitzen-van der Sluijs et al. (2016)
Belgium	Oost Vlaanderen	2015	<i>Salamandra salamandra</i>	210		210	Spitzen-van der Sluijs et al. (2016)
Belgium	Vlaams Brabant	2015	<i>Salamandra salamandra</i>	90		90	Spitzen-van der Sluijs et al. (2016)
Belgium	Antwerp	2015	<i>Ichthyosaura alpestris</i>	35	1	34	Spitzen-van der Sluijs et al. (2016)
Belgium	Oost Vlaanderen	2016	<i>Salamandra salamandra</i>	210		210	(3)
Belgium	Vlaams Brabant	2016	<i>Salamandra salamandra</i>	90		90	(3)
Belgium	Vlaams Brabant	2017	<i>Salamandra salamandra</i>	90		90	(3)
Belgium	Oost Vlaanderen	2017	<i>Salamandra salamandra</i>	210		210	(3)
Croatia	Dalmatia	2015	<i>Proteus anguinus</i>	14		14	(4)
Croatia	Istria	2015	<i>Proteus anguinus</i>	4		4	(4)
Croatia	Gorski Kotar	2015	<i>Proteus anguinus</i>	2		2	(4)
Croatia	Zagreb	2016	<i>Salamandra salamandra</i>	4		4	(4)
Czech Republic	Prague	2015	<i>Salamandra salamandra</i>	58		58	Baláz et al. (2018)
Czech Republic	Central Bohemian	2015	<i>Salamandra salamandra</i>	38		38	Baláz et al. (2018)
Czech Republic	Prague	2016	<i>Lissotriton vulgaris</i>	28		28	Baláz et al. (2018)
Czech Republic	Prague	2016	<i>Ichthyosaura alpestris</i>	2		2	Baláz et al. (2018)

Country	Region/ areas	Year	Species tested	Number of animals tested	Number of positive animals	Number of negative animals	Published/ acknowledged(*)
France	Cevenne (Cockle)	2015	<i>Salamandra salamandra</i>	30		30	⁽⁵⁾
Germany	Noordrijn Westfalen	2014	<i>Ichthyosaura alpestris</i>	5		5	Spitzen-van der Sluijs et al. (2016)
Germany	Noordrijn Westfalen	2014	<i>Salamandra salamandra</i>	19		19	Spitzen-van der Sluijs et al. (2016)
Germany	Noordrijn Westfalen	2014	<i>Lissotriton vulgaris</i>	19		19	Spitzen-van der Sluijs et al. (2016)
Germany	Saksen	2014	<i>Lissotriton vulgaris</i>	12		12	Spitzen-van der Sluijs et al. (2016)
Germany	Saksen	2014	<i>Ichthyosaura alpestris</i>	4		4	Spitzen-van der Sluijs et al. (2016)
Germany	Saksen	2014	<i>Salamandra salamandra</i>	15		15	Spitzen-van der Sluijs et al. (2016)
Germany	Noordrijn Westfalen	2015	<i>Salamandra salamandra</i>	167	84	83	Spitzen-van der Sluijs et al. (2016)
Germany	Rijnland palts	2015	<i>Salamandra salamandra</i>	55		55	Spitzen-van der Sluijs et al. (2016)
Germany	Rijnland palts	2015	<i>Ichthyosaura alpestris</i>	72		72	Spitzen-van der Sluijs et al. (2016)
Germany	Rijnland palts	2015	<i>Lissotriton helveticus</i>	1		1	Spitzen-van der Sluijs et al. (2016)
Germany	Beieren	2015	<i>Salamandra salamandra</i>	91		91	Spitzen-van der Sluijs et al. (2016)
Germany	Hessen	2015	<i>Salamandra salamandra</i>	69		69	Spitzen-van der Sluijs et al. (2016)
Germany	Beieren	2015	<i>Ichthyosaura alpestris</i>	1		1	Spitzen-van der Sluijs et al. (2016)
Germany	Rijnland palts	2015	<i>Lissotriton vulgaris</i>	15		15	Spitzen-van der Sluijs et al. (2016)
Germany	Nedersaksen	2015	<i>Salamandra salamandra</i>	23		23	Spitzen-van der Sluijs et al. (2016)
Germany	Saksen- anhalt	2015	<i>Salamandra salamandra</i>	8		8	Spitzen-van der Sluijs et al. (2016)
Germany	Noordrijn Westfalen	2015	<i>Ichthyosaura alpestris</i>	27		27	Spitzen-van der Sluijs et al. (2016)
Germany	Noordrijn Westfalen	2015	<i>Lissotriton vulgaris</i>	117		117	Spitzen-van der Sluijs et al. (2016)
Germany	Noordrijn Westfalen	2015	<i>Triturus cristatus</i>	27		27	Spitzen-van der Sluijs et al. (2016)
Germany	Thüringen	2015	<i>Triturus cristatus</i>	23		23	Spitzen-van der Sluijs et al. (2016)
Germany	Baden württemberg	2015	<i>Salamandra salamandra</i>	3		3	⁽⁵⁾
Portugal	Elisabeth	2015	<i>Lissotriton boscai</i>	8		8	⁽⁵⁾
Portugal	Varziela	2015	<i>Chioglossa lusitanica</i>	13		13	⁽⁵⁾
Portugal	Barreiro	2015	<i>Triturus marmoratus</i>	4		4	⁽⁵⁾
Portugal	Barreiro	2015	<i>Lissotriton boscai</i>	14		14	⁽⁵⁾
Portugal	Gorbelas	2015	<i>Salamandra salamandra</i>	1		1	⁽⁵⁾

Country	Region/ areas	Year	Species tested	Number of animals tested	Number of positive animals	Number of negative animals	Published/ acknowledged(*)
Portugal	Prados Garcia Renda	2015	<i>Lissotriton boscai</i>	3		3	(5)
Portugal	Prados Garcia Renda	2015	<i>Triturus marmoratus</i>	2		2	(5)
Portugal	Pena Rubia	2015	<i>Triturus marmoratus</i>	3		3	(5)
Portugal	Fraga Escuro	2015	<i>Lissotriton boscai</i>	5		5	(5)
Portugal	Castro Laboreiro	2015	<i>Lissotriton boscai</i>	6		6	(5)
Portugal	Castro Laboreiro	2015	<i>Triturus marmoratus</i>	3		3	(5)
Portugal	Rodeiro	2015	<i>Lissotriton boscai</i>	44		44	(5)
Portugal	Rodeiro	2015	<i>Triturus marmoratus</i>	14		14	(5)
Portugal	Lagoa da Peneda	2015	<i>Lissotriton boscai</i>	23		23	(5)
Portugal	Lagoa da Peneda	2015	<i>Triturus marmoratus</i>	22		22	(5)
Portugal	Podre	2015	<i>Lissotriton boscai</i>	12		12	(5)
Portugal	Podre	2015	<i>Triturus marmoratus</i>	5		5	(5)
Portugal	Curveira 01	2015	<i>Lissotriton boscai</i>	12		12	(5)
Portugal	Bago De Baixo	2015	<i>Triturus marmoratus</i>	20		20	(5)
Portugal	Bago De Baixo	2015	<i>Lissotriton boscai</i>	30		30	(5)
Portugal	Varzea Travessa	2015	<i>Triturus marmoratus</i>	33		33	(5)
Portugal	Varzea Travessa	2015	<i>Lissotriton boscai</i>	36		36	(5)
Portugal	Pena Rubia	2015	<i>Lissotriton boscai</i>	12		12	(5)
Portugal	Pena Rubia	2015	<i>Triturus marmoratus</i>	1		1	(5)
Portugal	Castro Laboreiro	2015	<i>Lissotriton boscai</i>	8		8	(5)
Portugal	Castro Laboreiro	2015	<i>Triturus marmoratus</i>	4		4	(5)
Portugal	Dorna	2015	<i>Lissotriton boscai</i>	1		1	(5)
Portugal	Dorna	2015	<i>Triturus marmoratus</i>	1		1	(5)
Portugal	Portella	2015	<i>Salamandra salamandra</i>	1		1	(5)
Slovenia	Barje	2017	<i>Triturus carnifex</i>	1		1	(6)
Slovenia	Barje	2017	<i>Lissotriton vulgaris</i>	2		2	(6)
Slovenia	Ljubljana	2017	<i>Rana dalmatina</i>	1		1	(6)
Slovenia	Ljubljana	2017	<i>Rana temporaria</i>	3		3	(6)
Slovenia	Ljubljana	2017	<i>Bufo bufo</i>	4		4	(6)
Slovenia	Ljubljana	2017	<i>Salamandra salamandra</i>	1		1	(6)
Slovenia	Barje (Bistra)	2017	<i>Lissotriton vulgaris</i>	1		1	(6)
Slovenia	Kranj	2017	<i>Proteus anguinus</i>	2		2	(6)
Slovenia	Ljubljana	2017	<i>Xenopus laevis</i>	2		2	(6)
Slovenia	Litija	2017	<i>Rana arvalis</i>	3		3	(6)
Slovenia	Mura	2017	<i>Rana arvalis</i>	7		7	(6)
Slovenia	Šmarje Sap	2017	<i>Bufo bufo</i>	1		1	(6)

Country	Region/ areas	Year	Species tested	Number of animals tested	Number of positive animals	Number of negative animals	Published/ acknowledged(*)
Slovenia	Ig	2017	<i>Lissotriton vulgaris</i>	1		1	(6)
Slovenia	Ljubljana	2017	<i>Triturus carnifex</i>	3		3	(6)
Slovenia	Vipava	2017	<i>Proteus anguinus</i>	3		3	(6)
Slovenia	Ljubljana	2017	<i>Proteus anguinus</i>	5		5	(6)
Spain	Picos de Europa National Park	2016	<i>Ichthyosaura alpestris</i>	82		82	(1)
Spain	Sierra de Guadarrama National Park	2016	<i>Ichthyosaura alpestris</i>	27		27	(1)
Spain	Alpedrete	2016	<i>Triturus pygmaeus</i>	13		13	(1)
Spain	Pyrenees	2016	<i>Calotriton asper</i>	53		53	(1)
Spain	Montseny Natural Park	2016	<i>Calotriton arnoldi</i>	11		11	(1)
Spain	Doñana National Park	2016	<i>Lissotriton boscai</i>	18		18	(1)
Spain	Doñana National Park	2016	<i>Pelobates cultripes</i>	2		2	(1)
Spain	Doñana National Park	2016	<i>Pleurodeles waltl</i>	7		7	(1)
Spain	Doñana National Park	2016	<i>Triturus pygmaeus</i>	126		126	(1)
Spain	Montseny Natural Park	2017	<i>Rana temporaria</i>	2		2	(1)
Spain	Montseny Natural Park	2017	<i>Bufo spinosus</i>	4		4	(1)
Spain	Montseny Natural Park	2017	<i>Calotriton arnoldi</i>	32		32	(1)
Spain	Montseny Natural Park	2017	<i>Triturus marmoratus</i>	2		2	(1)
Spain	Soncillo	2017	<i>Salamandra salamandra</i>	5		5	(1)
Switzerland	Lepontine alps	2008–2013	<i>Ichthyosaura alpestris</i>	10		10	Parrot et al. (2017)
Switzerland	Lepontine alps	2008–2013	<i>Salamandra salamandra</i>	2,223		2,223	Parrot et al.,(2017)
Switzerland	Lepontine alps	2008–2013	<i>Triturus carnifex</i>	22		22	Parrot et al.,(2017)
Switzerland	Lepontine alps	2008–2013	<i>Lissotriton vulgaris</i>	10		10	Parrot et al.,(2017)
Netherlands	Limburg	2010	<i>Salamandra salamandra</i>	6	6		Martel et al.,(2013)
Netherlands	Limburg	2013	<i>Ichthyosaura alpestris</i>	1		1	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2013	<i>Salamandra salamandra</i>	57		57	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2014	<i>Ichthyosaura alpestris</i>	49	1	48	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2014	<i>Lissotriton vulgaris</i>	12		12	Spitzen-van der Sluijs et al. (2016)

Country	Region/ areas	Year	Species tested	Number of animals tested	Number of positive animals	Number of negative animals	Published/ acknowledged(*)
Netherlands	Limburg	2014	<i>Lissotriton helveticus</i>	24		24	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2014	<i>Salamandra salamandra</i>	43		43	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2015	<i>Ichthyosaura alpestris</i>	225	37	188	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2015	<i>Lissotriton vulgaris</i>	76	65	11	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2015	<i>Lissotriton helveticus</i>	18		18	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2015	<i>Salamandra salamandra</i>	32		32	Spitzen-van der Sluijs et al. (2016)
Netherlands	Gelderland	2015	<i>Ichthyosaura alpestris</i>	8	8		Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2016	<i>Salamandra salamandra</i>	2		2	Spitzen-van der Sluijs et al. (2016)
Netherlands	Limburg	2017	<i>Ichthyosaura alpestris</i>	97		97	Spitzen-van der Sluijs et al. (2018)
Netherlands	Limburg	2017	<i>Salamandra salamandra</i>	35		35	Spitzen-van der Sluijs et al. (2018)
Netherlands	Limburg	2017	<i>Lissotriton vulgaris</i>	36		36	Spitzen-van der Sluijs et al. (2018)
Netherlands	Limburg	2017	<i>Lissotriton helveticus</i>	4		4	Spitzen-van der Sluijs et al. (2018)
Netherlands	Gelderland	2017	<i>Triturus cristatus</i>	2		2	Spitzen-van der Sluijs et al. (2018)
Netherlands	Noord Holland	2017	<i>Lissotriton vulgaris</i>	8		8	Spitzen-van der Sluijs et al. (2018)
Netherlands	Gelderland	2017	<i>Ichthyosaura alpestris</i>	1		1	Spitzen-van der Sluijs et al. (2018)
Netherlands	Drenthe	2017	<i>Triturus cristatus</i>	1		1	Spitzen-van der Sluijs et al. (2018)
Netherlands	Zuid Holland	2017	<i>Lissotriton vulgaris</i>	8		8	Spitzen-van der Sluijs et al. (2018)
Netherlands	Noord Holland	2017	<i>Lissotriton vulgaris</i>	1		1	Spitzen-van der Sluijs et al. (2018)

(*): When the data are still unpublished, the data providers are cited and acknowledged:

(1): J. Bosch, C. Monsalve and C. Sausor, CSIC, Spain.

(2): F. Glaser, M. Grabher, P. Kaufmann, G. Ludwig, A. Maletzky, G. Munimanda, G. Pechlaner, D. Preininger, S. Smith, K. Smole-Wiener, S. Schweiger, T. Wampula, C. Walzer and volunteers. Department of Integrative Biology and Evolution, University of Veterinary Medicine Vienna and Schönbrunn Zoo, Austria.

(3): A. Martel, F. Pasmans, Ghent University, Belgium, research funded by ANB (Flemish government).

(4): M. Lukac, Faculty of Veterinary Medicine, University of Zagreb, Croatia.

(5): D. Schmeller, UFZ, Germany.

(6): R. Kostanjsek and N. Gunde Cimerman, Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia.

Appendix C – Individual experts' assessments²⁹

Expert knowledge was elicited in two steps first asking for the central tendency and second for range parameters of the uncertainty distribution according to the following protocol:

Please assess the following list of mitigation measures according to their

C.1. Part I – Judgement

Please first assess the two measures on the scale between 'rubbish' (impractical, negligible) to 'fantastic' (easy-go, very high). The extreme categories are narratively described in the questionnaire. During the judgement integrate across multiple aspects e.g. balancing cost issues, personal resource needs and possible long-term sustainability. It is expected that you present your own expert view.

Please mark, e.g. by an 'I' where you want to set the mark.

Please now do the judgement across the table (preferably row by row). It is ok while judging to compare with your other judgement outcomes. If you think you do not know the answer, please still put a best guesstimate (which you can later accompany with maximum uncertainty making your input not influencing the outcome while keeping the uncertainty)

Example: o-----i-----o

C.2. Part II – Uncertainty

After having marked your best guesstimate judgement on the interval scale, you are asked to associate each judgement with an uncertainty rating. The intention is to express the confidence you have post hoc into your rating. To carry out this, we will perform two steps on each judgement:

Step A: First, the most extreme implication of your uncertainty is provided by marking the minimal and maximal value, right and left of your main judgement, for which you would think the feasibility/effectiveness could be considered barely possible (even if you would be extremely astonished if it actually would be that extreme). The 'm's should be placed left and right of – but not necessarily symmetrically to – the main judgement.

Step B: Now please mark another two values e.g. 'I' (one left and one right of the main judgement and in-between the two 'm's) that would indicate roughly the section of the interval in which you, according to your uncertainty reflection, would expect the most adequate feasibility/effectiveness score with at least 50% certainty – that's the interval you would fairly expect to find the adequate feasibility/effectiveness score if you would, e.g. know more new facts addressing your uncertainty/knowledge gaps. The two 'I's should be placed left and right of – but not necessarily symmetrically to – the main judgement; and in-between the two 'm's.

Example: o----m-----|---i---m-----o

Feasibility was assessed on a continuous scale from 'least feasible' to 'most feasible'.

'Least feasible': implementation demanding extensive resources e.g. it requires lots of personnel (qualified personnel handling most of the individuals), large amount of money (expensive for the reagents and facilities; complexity of monitoring the conduct), danger to the environment (for other species, e.g. causing toxicity or resistance).

'Most feasible': implementation demanding few resources for number of personnel (it does not require additional qualified personnel), amount of money (cheap reagents and existing facilities/does not require reagents and facilities) and implying negligible danger to the environment.

'Effectiveness' was assessed on a continuous scale from 'negligible' to 'fully protective'.

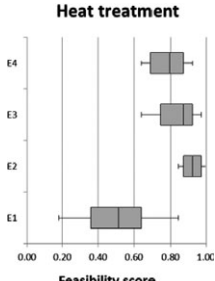
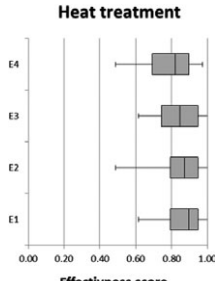
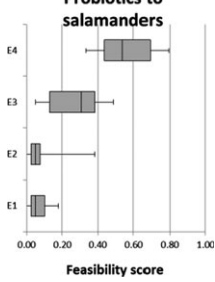
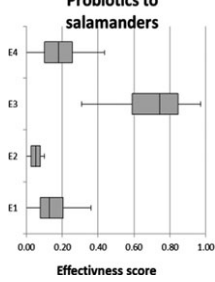
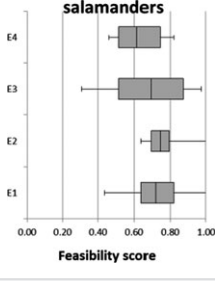
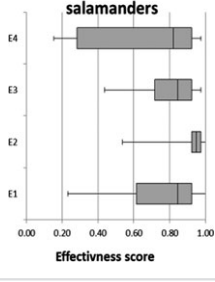
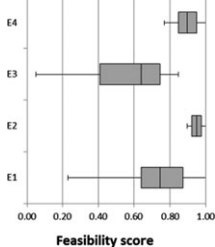
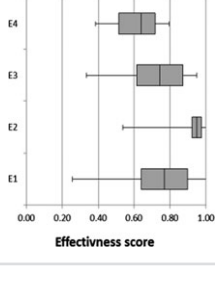
'Negligible' for kept salamanders means that the activity does not alter the exposure to Bsal introduction to uninfected populations or the further perpetuation between salamanders within an infected population; for wild salamanders, it means that the activity does not alter the exposure to Bsal introduction of an uninfected site or the further perpetuation between salamanders on an infected site.

'Fully protective': the activity leads to refractory protection of uninfected salamanders in a kept population against Bsal infection, or to refractory protection of uninfected wild salamanders on a site against Bsal infection (for on-site protection).

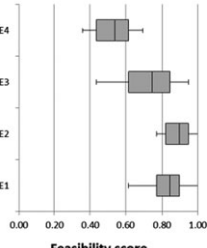
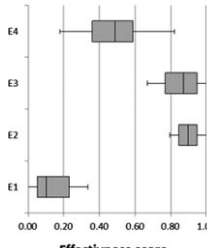
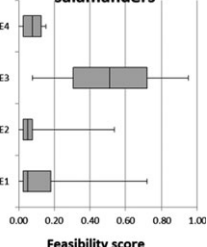
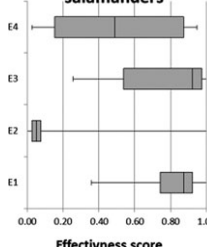
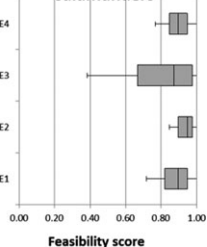
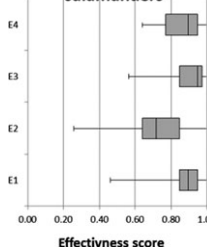
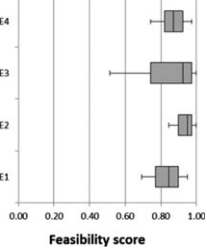
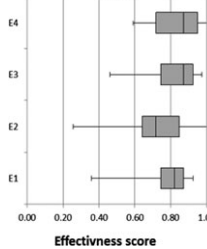
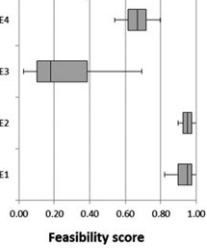
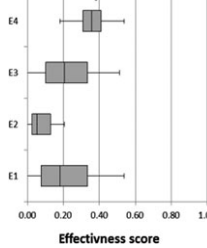
²⁹ E1 to E3 are working actively with Bsal or Bd.

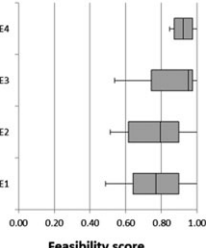
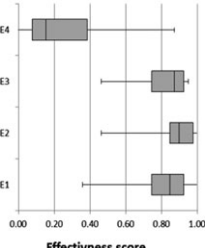
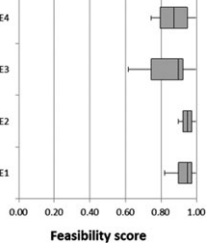
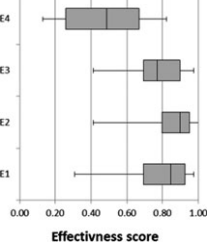
C.3. Assessment of the feasibility and effectiveness of the risk-mitigating measures for safer international or intra-EU trade of salamanders (ToR 3b)

C.3.1. Individual assessment of the feasibility and effectiveness³⁰

#	Risk-mitigation measures	Definition/explanation	Score for feasibility integrating human resources, technical efforts, treatment costs and environmental side-effects	Score for effectiveness in preventing translocation of pathogen along those pathways the mitigation measure is meant to address
1	Preventative heat treatment	To treat animals in an environment at 25°C for 10 days (see Section 3.2.1.2)		
2	Quarantine	Previously reported in Grant et al. (2016) Keep traded salamanders in tanks at entrance point for the whole Bsal incubation time while testing them for Bsal (see Section 3.1.2.1) over the last week		
3	Require pre-movement health certification	Previously reported in Grant et al. (2016) Individual Bsal test of salamanders (see Section 3.1.2.1)		
4	Bsal testing at the entry point	Keep traded salamanders in tanks at entrance point while individual testing for Bsal (see Section 3.1.2.1). Positive animals will be treated (according to protocols explained in Section 3.2.1.1)		

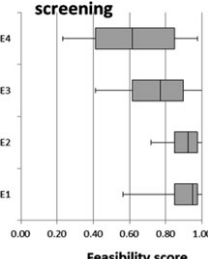
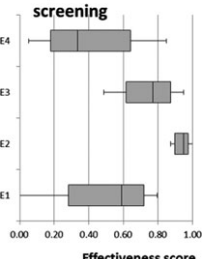
³⁰ Expert knowledge is elicited using distributional parameters, i.e. most plausible as central tendency, barely possible values as minimum and maximum (whiskers) and the central 50% interval of certainty (grey box). Data shown are before consensus discussion.

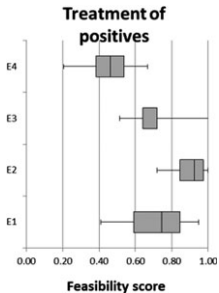
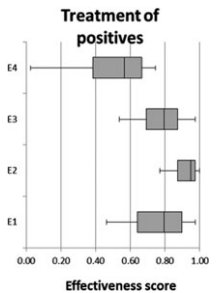
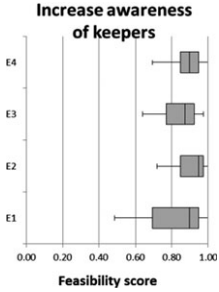
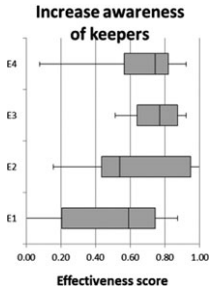
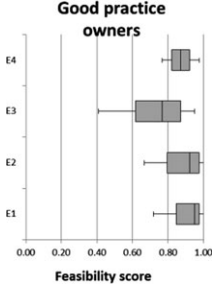
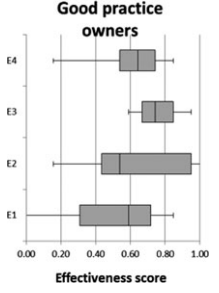

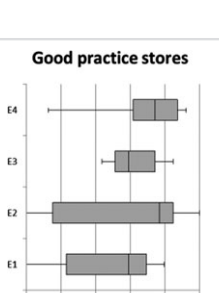
#	Risk-mitigation measures	Definition/explanation	Score for feasibility integrating human resources, technical efforts, treatment costs and environmental side-effects	Score for effectiveness in preventing translocation of pathogen along those pathways the mitigation measure is meant to address
5	Restrict importation of some salamanders	Previously reported in Grant et al. (2016) Restrictions/partial ban of salamander introduction on the basis of the origin or for some salamander families	<p>Test at entry point</p>  <p>Feasibility score</p>	<p>Test at entry point</p>  <p>Effectiveness score</p>
6	Ban all importation of salamanders	Previously reported in Grant et al. (2016) Ban of all salamanders trade (Martel et al., 2014; Yap et al., 2015; Berger et al., 2016; Richgels et al., 2016)	<p>Vaccinate salamanders</p>  <p>Feasibility score</p>	<p>Vaccinate salamanders</p>  <p>Effectiveness score</p>
7	Tracking all traded species	Use an unique code for identifying salamanders' (or amphibians' in general) shipments (Auliya et al., 2016; EFSA, 2017)	<p>Ban importation of salamanders</p>  <p>Feasibility score</p>	<p>Ban importation of salamanders</p>  <p>Effectiveness score</p>
8	Hygiene procedures	Equipment that enters in contact with salamanders should be cleaned and disinfected (according to the protocols reported in Section 3.2.1) (EFSA, 2017)	<p>Restrict salamander trade</p>  <p>Feasibility score</p>	<p>Restrict salamander trade</p>  <p>Effectiveness score</p>
9	Good practice manuals	Draw up and spread a good practice code containing guidelines for traders for handling salamanders and fomites (Murray et al., 2011b)	<p>Tracking traded species</p>  <p>Feasibility score</p>	<p>Tracking traded species</p>  <p>Effectiveness score</p>

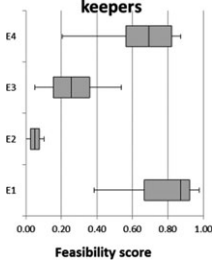
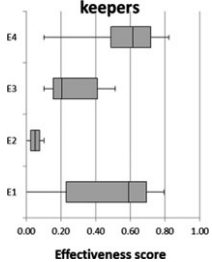
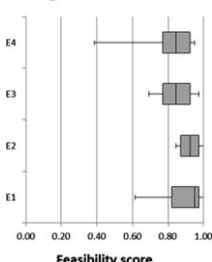
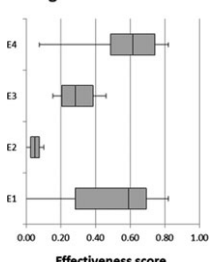
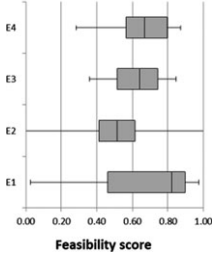
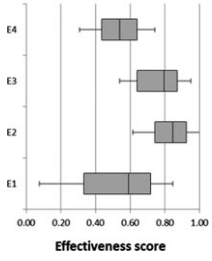
#	Risk-mitigation measures	Definition/explanation	Score for feasibility integrating human resources, technical efforts, treatment costs and environmental side-effects	Score for effectiveness in preventing translocation of pathogen along those pathways the mitigation measure is meant to address
10	Apply probiotics to salamanders	Previously reported in Grant et al. (2016) Preventative probiotic in salamanders' skin. The method has not been set up for Bsal Examples of studies on Bd are reviewed in Bletz et al. (2013)	<p>Hygienic procedures</p>  <p>Feasibility score</p>	<p>Hygienic procedures</p>  <p>Effectiveness score</p>
11	Preventative heat treatment	To treat animals in an environment at 25°C for 10 days (see Section 3.2.1.2)	<p>GP handling traded animals and fomites</p>  <p>Feasibility score</p>	<p>GP handling traded animals and fomites</p>  <p>Effectiveness score</p>

C.4. Assessment of the feasibility and effectiveness of the potential risk-mitigating measures for the protection of kept salamanders (ToR 4a)

C.4.1. Individual assessment of the feasibility and effectiveness³⁰

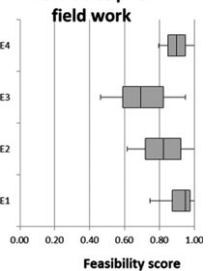
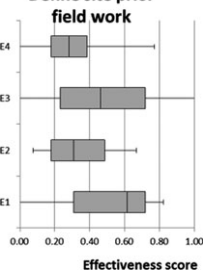
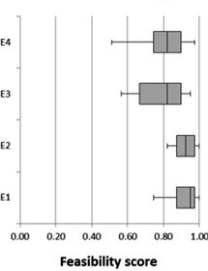
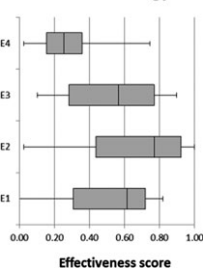
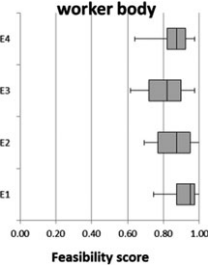
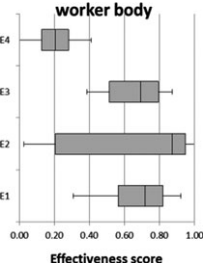
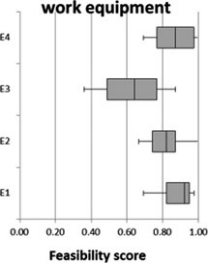
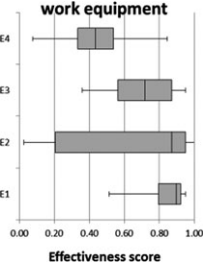
#	Risk-mitigation measures	Definition/explanation in place in the EU (e.g.)	Score for feasibility of measures in KEPT salamanders integrating human resources, stakeholder willingness to comply/implement, technical complexity, feasibility of monitoring, treatment costs and long-term outlook, e.g. permanent efforts	Score for effectiveness in protecting KEPT salamanders against Bsal infection
1	Screening of the kept populations	Test skin swabs from captive kept salamanders for presence of Bsal (see Section 3.1.2.1) In place as non-systematic screening: BE (research funding) CZ (research funding) DE (research funding) NL (state funding) UK (research funding)	<p>Population screening</p>  <p>Feasibility score</p>	<p>Population screening</p>  <p>Effectiveness score</p>

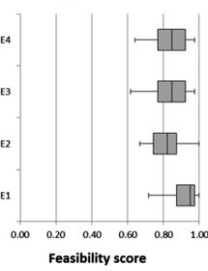
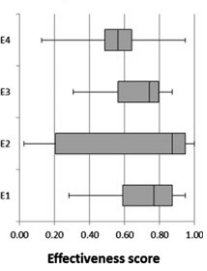
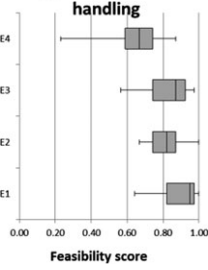
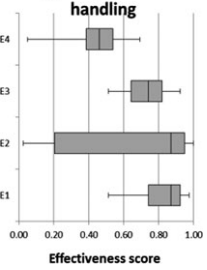
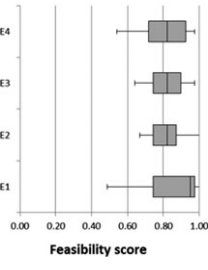
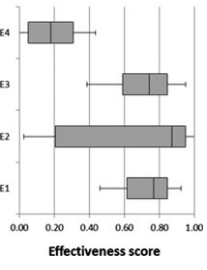
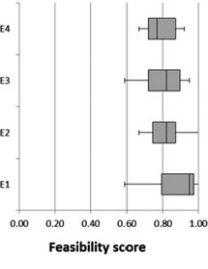
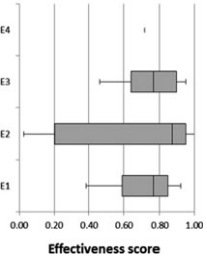
#	Risk-mitigation measures	Definition/explanation in place in the EU (e.g.)	Score for feasibility of measures in KEPT salamanders integrating human resources, stakeholder willingness to comply/implement, technical complexity, feasibility of monitoring, treatment costs and long-term outlook, e.g. permanent efforts	Score for effectiveness in protecting KEPT salamanders against Bsal infection
2	Treatment of the positive cases	Treatment of the Bsal-positive salamanders (see Section 3.2.1) In place: BE (private funding) DE (research funding, private funding) NL (research funding, private funding)	 <p>Feasibility score</p>	 <p>Effectiveness score</p>
3	Increase in owners' awareness	Disease information and information on good practices for keepers, breeders and stores via multiple tools (e.g. Internet, advertisements, oral presentations at meetings, social media) In place in: BE, FR, DE, IT, ES, NL, UK	 <p>Feasibility score</p>	 <p>Effectiveness score</p>
4	Good practices and hygiene protocols for keepers and breeders	Develop good practices manuals and written guidelines with hygiene protocols for correct management of salamanders in captivity, the equipment they are in contact with and the environment For keepers and breeders of salamanders In place in a certain extent in: BE, FR, DE, IT, ES, NL, UK	 <p>Feasibility score</p>	 <p>Effectiveness score</p>
5	Good practices and hygiene protocols for the stores/pet-shops	As above, for the pet-shops and stores selling salamanders Not in place yet	 <p>Feasibility score</p>	 <p>Effectiveness score</p>

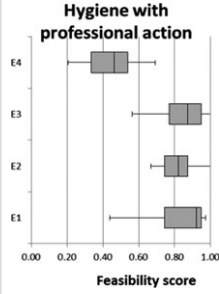
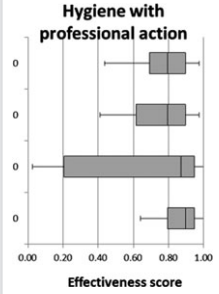
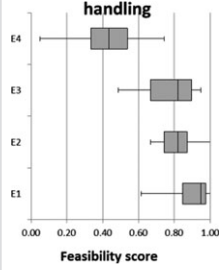
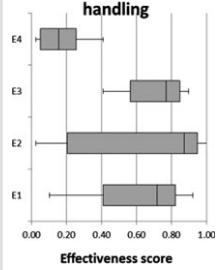
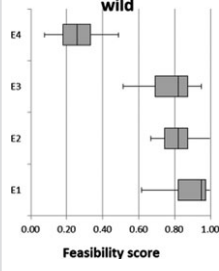
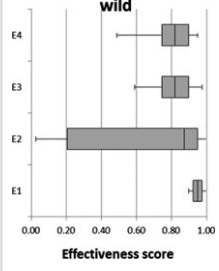
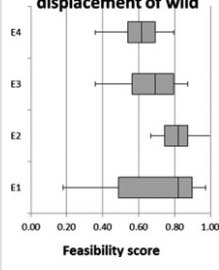
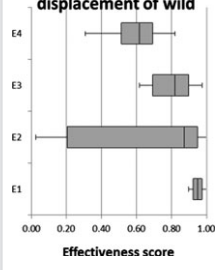
#	Risk-mitigation measures	Definition/explanation in place in the EU (e.g.)	Score for feasibility of measures in KEPT salamanders integrating human resources, stakeholder willingness to comply/implement, technical complexity, feasibility of monitoring, treatment costs and long-term outlook, e.g. permanent efforts	Score for effectiveness in protecting KEPT salamanders against Bsal infection
6	Registration of the keepers/breeders of salamanders	Registration of keepers' and breeders' facilities with an indication of at least: (i) where keepers are located, (ii) the species that are kept (at least for the main families kept), and (iii) the range of number of animals that are kept (e.g. 1–10; 10–100; > 100) Not in place yet	Registration of keepers 	Registration of keepers 
7	Registration of the stores/pet-shops of salamanders	Registration of the stores and pet-shops selling salamanders with indication of at least: (i) where they are located, (ii) the main species that they sell (at least at a family level), (iii) the maximum amount of salamanders that their facilities can keep	Registration of stores 	Registration of stores 
8	Education and training of stakeholders	Organise training courses on correct keeping and managing amphibians for keepers, breeders, stores, pet-shops Not in place yet	Education & training 	Education & training 

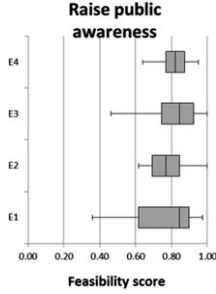
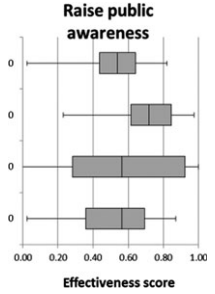
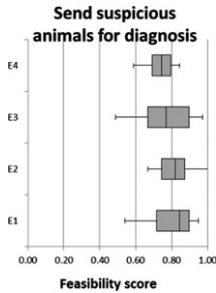
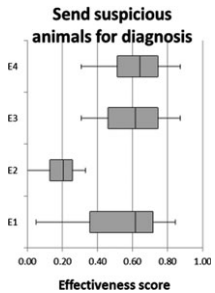
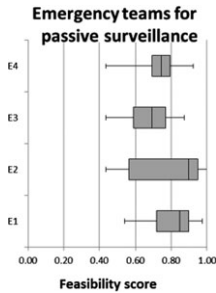
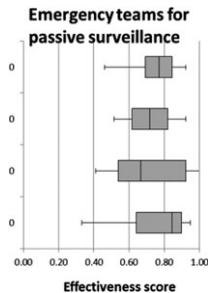
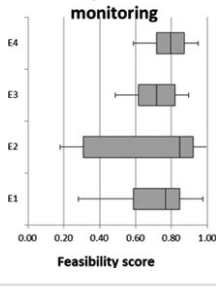
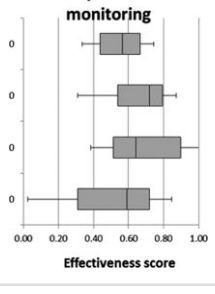
C.5. Assessment of the feasibility and effectiveness of the potential risk-mitigating measures for on-site protection of wild salamanders in their natural habitats (ToR 4b)

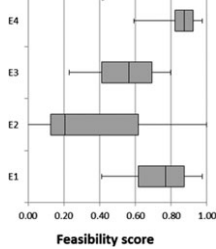
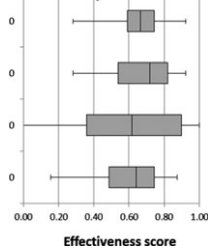
C.5.1. Individual assessment of the feasibility and effectiveness³⁰

#	Risk-mitigation measures	Definition/explanation in place in the EU (e.g.)	Score for feasibility of measures in WILD salamanders integrating human resources, stakeholder willingness to comply/implement, technical complexity, feasibility of monitoring, treatment costs and long-term outlook, e.g. permanent efforts	Score for effectiveness in protecting WILD salamanders against Bsal infection
1	Site definition	Defining a site robustly to identify opportunities for disease spread between sites	<p>Define site prior field work</p>  <p>Feasibility score</p>	<p>Define site prior field work</p>  <p>Effectiveness score</p>
2	Site visitation strategy	Defining a site visitation strategy when attending multiple sites in sequence	<p>Site visit strategy</p>  <p>Feasibility score</p>	<p>Site visit strategy</p>  <p>Effectiveness score</p>
3	Cleaning and disinfection of field worker body parts	Cleaning and washing or wiping with a suitable disinfectant (see Section 3.2.1) of hands, arms, knees, etc., before a site is entered and/or after finishing work at a site	<p>Desinfection field worker body</p>  <p>Feasibility score</p>	<p>Desinfection field worker body</p>  <p>Effectiveness score</p>
4	Cleaning and disinfection of field equipment	Cleaning and disinfection of footwear and equipment (e.g. vehicles, nets, balances) at the commencement of fieldwork and between each sampling site	<p>Desinfection field work equipment</p>  <p>Feasibility score</p>	<p>Desinfection field work equipment</p>  <p>Effectiveness score</p>

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5	Use disposable items	Use of disposable items (e.g. single-use latex, nitrile or vinyl gloves) for moving and handling salamanders	<p>Disposable items</p>  <p>Feasibility score</p>	<p>Disposable items</p>  <p>Effectiveness score</p>
6	Care in handling salamanders	If handling is essential, ensure that individuals do not have their exposure to pathogens elevated over their background exposure levels	<p>Hygienic protocol if handling</p>  <p>Feasibility score</p>	<p>Hygienic protocol if handling</p>  <p>Effectiveness score</p>
7	Hands disinfection	If gloves for handling animals are unavailable, disinfect hands with 70% ethanol and wash hands thoroughly in water after treatment. If ethanol is not available, the minimum treatment is hand washing in the water to which the amphibian is usually exposed	<p>Hands disinfection</p>  <p>Feasibility score</p>	<p>Hands disinfection</p>  <p>Effectiveness score</p>
8	Single use of containers for temporarily holding salamanders	For temporarily held animals, ensure single-use or disinfected containers between each animal. Adults should not be held in groups	<p>Single-use containers</p>  <p>Feasibility score</p>	<p>Single-use containers</p>  <p>Effectiveness score</p>

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9	Integrated hygiene approach	An integrated approach to prevent or limit disease transmission includes all the single measures for hygiene procedures and good practices outlined above (from ex-3 to ex-9, no ex-6)	 <p>Hygiene with professional action</p> <p>Feasibility score</p>	 <p>Hygiene with professional action</p> <p>Effectiveness score</p>
10	Avoid wild salamanders capture and handling	Avoiding or minimising the capture, handling and housing of wild salamanders helps reduce or remove anthropogenic sources of disease transmission between animals and introduction/spread from one place to another	 <p>No wild capture & handling</p> <p>Feasibility score</p>	 <p>No wild capture & handling</p> <p>Effectiveness score</p>
11	Prevent translocation of wild animals	Treating translocated amphibians as if they are infected can help mitigate the risk of intentional translocation and therefore disease spread	 <p>Prevent return to wild</p> <p>Feasibility score</p>	 <p>Prevent return to wild</p> <p>Effectiveness score</p>
12	Prevent return of captive animals to the wild	Wild amphibians and their larvae that need to be housed for some time in a captive situation (e.g. laboratory, zoo or captive breeding facilities) should not be returned to the wild. The measure includes increasing awareness of salamanders' keepers that should not release kept salamanders into the wild	 <p>Prevent displacement of wild</p> <p>Feasibility score</p>	 <p>Prevent displacement of wild</p> <p>Effectiveness score</p>

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13	Increase public awareness and participation	Disease information and education campaigns to promote participation and enforce hygiene protocols and good practices via multiple tools (e.g. installation of signage for people visiting natural areas and producing informational flyers). It includes facilitating reporting of signs/symptoms (e.g. sighting for invasive species), and means to communicate observations of sick or dead animals (e.g. via creation of hotlines)	 <p>Feasibility score</p>	 <p>Effectiveness score</p>
14	Collect and send dead animals for Bsal diagnosis	As a part of passive surveillance, dead amphibians should be collected and sent for disease diagnosis	 <p>Feasibility score</p>	 <p>Effectiveness score</p>
15	Set up wildlife disease emergency teams for passive surveillance	A contact for reporting and consultation in the case a dead salamander is found. This measure includes a laboratory where testing salamanders for Bsal (see Section 3.1.2.1), reporting the Bsal-positive animals and accompanying relevant information to the local authorities can be conducted (see Section 3.1.2.2)	 <p>Feasibility score</p>	 <p>Effectiveness score</p>
16	Wild population monitoring	To collate and retrieve data on population sizes, distributions and trends	 <p>Feasibility score</p>	 <p>Effectiveness score</p>

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17	Active surveillance	To actively collect data on Bsal presence in amphibian populations by visiting and screening for disease local populations of salamanders according to a clearly defined and harmonised sampling strategy (see Sections 3.1.2.1 and 3.1.2.2)	<p>Active surveillance for early detection</p>  <p>Feasibility score</p>	<p>Active surveillance for early detection</p>  <p>Effectiveness score</p>